nano-tera.ch
swiss scientific initiative in health / security / environment systems

ISyPeM II | RTD 2015

FNSNF

Hes·so VALAIS WALLIS
School of Engineering

Drug quantification in blood within microstructures for Point-of-Care Therapeutic Drug Monitoring

Diana Burghelea, Denis Prim, Helene Strese, Frederic Truffer
Martial Geiser, Marc Pfeifer, Jean-Manuel Segura
School of Engineering, HES-SO Valais - Wallis
Route du Rawyl 64, CH-1950 Sion, Switzerland

THERAPEUTIC DRUG MONITORING

Therapeutic Drug Monitoring (TDM) allows for personalized dosage during patients' therapeutic treatments. It is often mandatory for modern potent drugs against cancer, HIV or in organ transplantation cases.

Issues: Curently, this process is demanding for the patient, slow and costly as it is performed in central analytical laboratories using ml of blood.

In pediatrics for example, where the amount of sample available for collection is minimal and the feedback time is extremely important for proper clinical decisions, a simple, rapid and sensitive solution is needed.

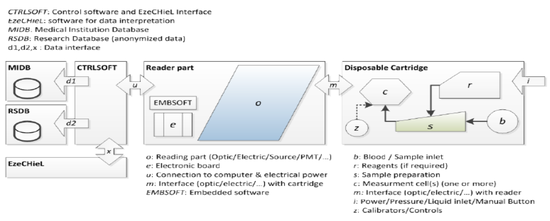


Fig 1: Product overview

Aim: to develop a compact and cost-effective Point-Of-Care (POC) drug quantification device, based on miniaturized competition immunoassays.

Approach: Integration into an automized device of key steps like sample preparation, reagent flow, mixing, reaction and quantitative measurement of small molecules.

IMMUNOASSAY APPROACH

Drug quantification was performed using Fluorescence Polarization Immunoassays **(FPIA),** which is currently the method of choice in clinical laboratories due to its sensitivity, specificity and cost effectiveness.

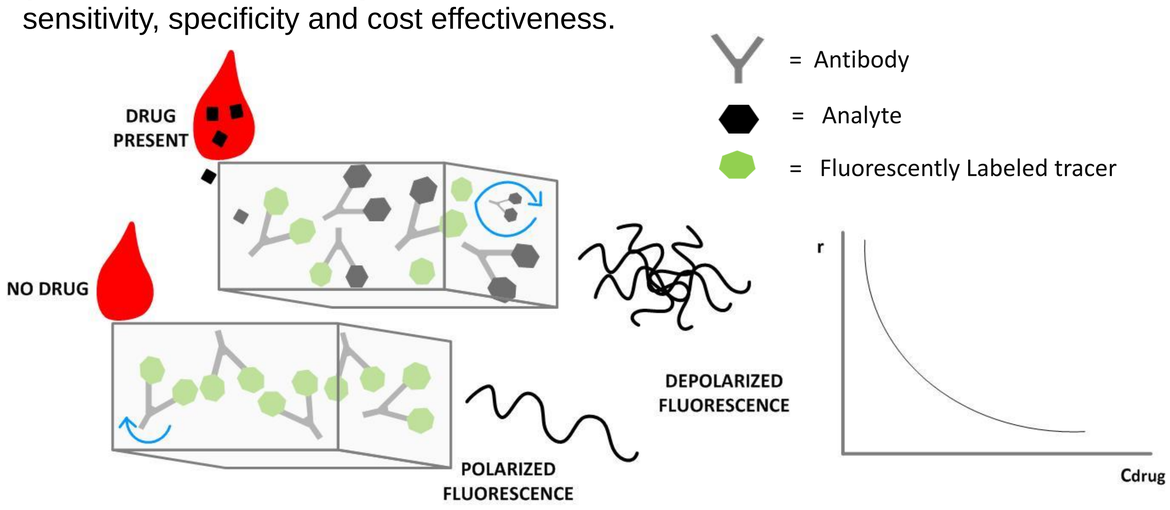


Fig 2: Principle of FPIA implemented in microstructures for small molecule quantification. The analyte present in the blood drop and a fluorescent labeled derivative, deposited in the microstructure, are competing for the binding sites of the antibodies. The concomitent replacement of tracer by drug at the specific sites of the antibody causes a decrease in fluorescence.

1 MINIATURIZATION OF FPIA

- ✓ Tobramycin is an aminoglycoside antibiotic used against bacterial infections often prescribed to neonates and children and requires special attention to control variations of its concentration in the body.
- ✓ Tobramycin could be quantified with a novel FP assay with high precision (within-run CV < 10%) and accuracy (recoveries between 90 and 110%) in the therapeutic range of 1 to 10 µg/ml.
- ✓ LOQ and LOD are 0.2 and 0.6 µg/ml, respectively. It typically requires only 10 µl of serum and could be further downsized to just one µl of serum which demonstrates its aptitude for point-of-care therapeutic drug monitoring.

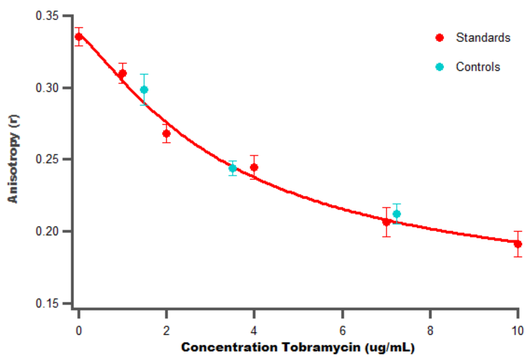
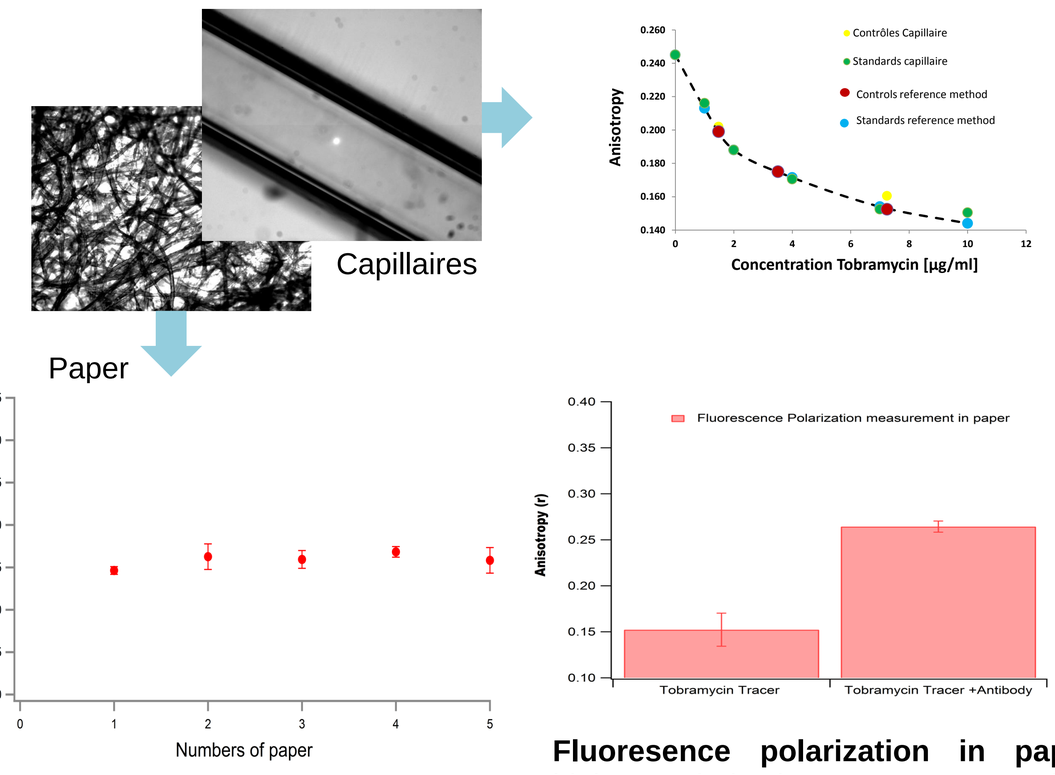


Fig 3: FPIA Calibration curve with a novel derivative for Tobramycin quantification using minute amounts of blood

2 FPIA INTEGRATION INTO MICROSTURCTURES

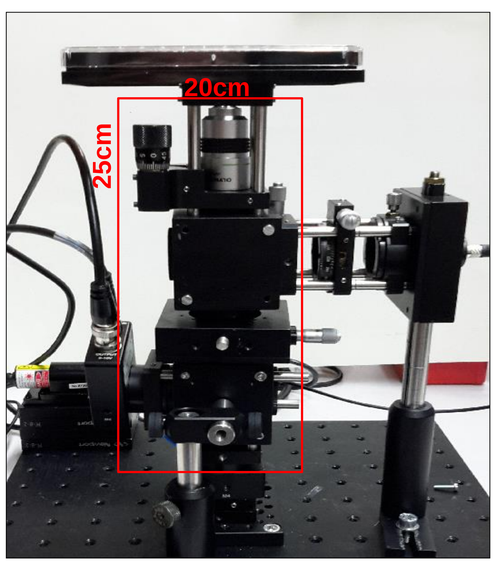
- ✓ Tobramycin could efficiently be quantified, from minute amounts of blood, in capillaries and paper;



Reproducibilty of measurements in paper

Fluoresence polarization in paper: higher polarization was measured in paper when the drug derivative was bounded to the antibody due to the increased size of the complex.

3 PRELIMINARY POC-TDM PROTOTYPE



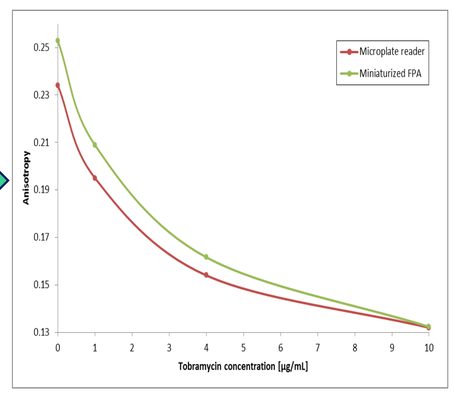
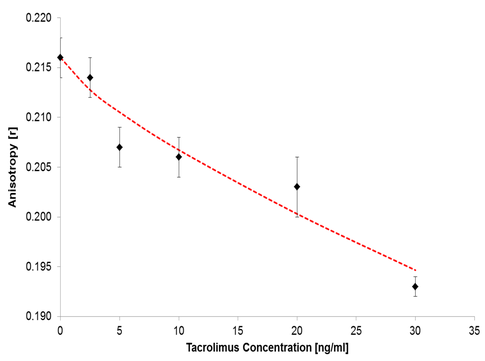


Fig.3: Tobramycin concentration could be efficiently quantified on a first compact optical **prototype.**

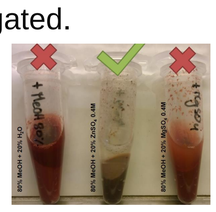
4 NOVEL TACROLIMUS QUANTIFICATION

For Tacrolimus, a challenging target due to its very **narrow therapeutic range,** (6 -25 nM), and its **hydrophobicity**, we achieved the establishment of a preliminary FP immunoassay with reduced performance.



Miniaturized Fluorescent Immunoassay for the quantification of Tacrolimus from whole blood

The 'Trouble drop' of whole blood: The target of Tacrolimus inside the red blood cells makes its quantification difficult. Challenging sample preparation in terms of **lysis**, **separation**, **extraction** followed by the FP **quantification** of the drug is currently investigated.



CONCLUSIONS

- ✓ FPIA miniaturized assays can be transferred into glass and paper-based microstructures;
- ✓ A Point-of-Care Therapeutic Drug Monitoring prototype enabled small molecule quantification.

CONTACTS

Prof Dr. Marc Pfeifer: marc.pfeifer@hevs.ch
Prof Dr. Jean-Manuel Segura: jmanuel.segura@hevs.ch

Valais excellence CERTIFIED