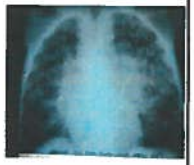
Tuberculosis POCT: An Integrated Photonic Biosensor for Tuberculosis Detection

Ayssar A. Elamin, D. Martens, A. B. González-Guerrero, M. Stehr, F. Jonas, W. Van Roy, R. Vos, A. Stassen, S. Severi, R. Bockstaele, P. Soetaert, B. Anton, H. Becker, L. M. Lechuga, P. Bienstman, Mahavir Singh

Goal: Point of care test for tuberculosis

- In 2014, TB killed 1.5 million people (1.1 million HIV-negative and 0.4 million HIV-positive).
- In 2014, 6 million new cases of TB
- TB now ranks alongside HIV as a leading cause of death worldwide.

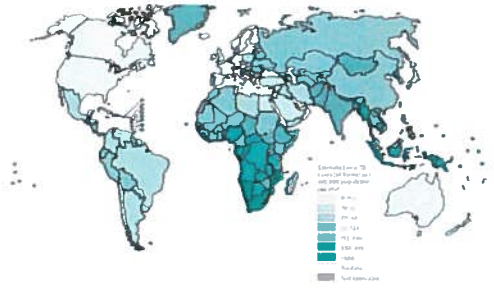
- Resistance
  - MDR-TB (3.3% of new TB cases and 20% of previously treated cases have MDR-TB)
  - XDR-TB
  - TDR-TB



- Burden of latent infection

Goal: Point of care test for tuberculosis

- Tuberculosis is a global disease
- Current methods for the detection of TB are either time consuming or require expensive instruments (not point of care) and no rapid tests for diagnosis of active TB are available
- **World market for Tuberculosis diagnosis: more than 1 billion US$**
- **HIV and TB is a deadly combination killing the maximum number of people world-wide due to any infectious diseases**

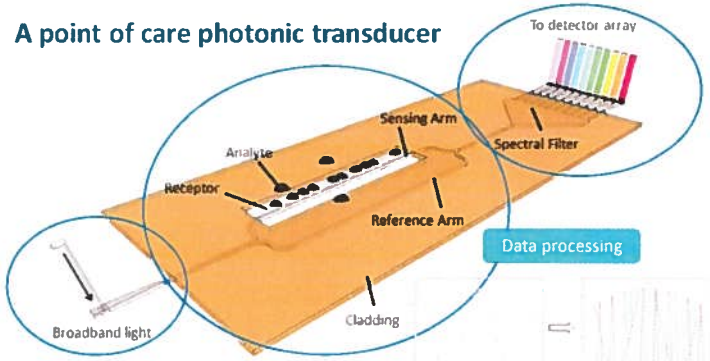


Estimated TB incidence rates, 2014 (WHO, Global tuberculosis report (2015))

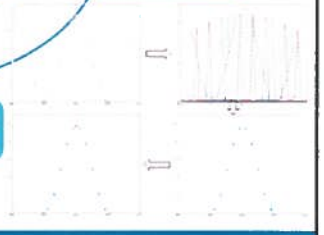
Progress beyond state-of-the-art in TB detection

Culture test (BACTEC MGIT)	Nucleic acid test (Cepheid GenXpert)	Lab-based ELISA Serolgy	Lateral flow tests
			
- Gold standard	- Drug resistance information	- Cheap (1-2 euros)	- Cheap (2 euros)
- Costly - Needs instrumentation and a lab - Time consuming	- Costly (20 euros) - Unsuited for tropical, TB-endemic countries	- Sensitivity<60% - Trained personnel - No drug resistance information	- Low sensitivity (30-60%) and specificity (60-90%) -Blood test, urine?? - No drug resistance information

A point of care photonic transducer



Silicon nitride waveguides to use 850 nm light for reduced water absorption



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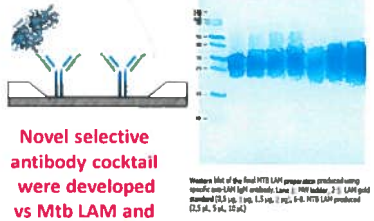
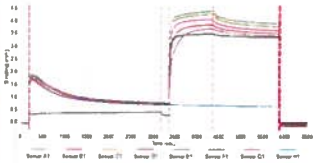
Novel and highly selective TB biomarker developed





Novel selective antibody cocktail were developed vs Mtb LAM and Ag85 complex

Kinetics characterization of one monoclonal anti-LAM antibody using APS sensors on octet platform

High-quality antibodies with KD around $10^{-8} – 10^{-9}$

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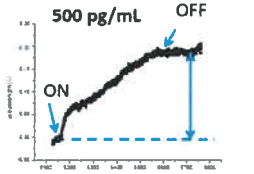
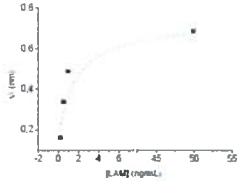
Preliminary detection of LAM with photonic biosensor

- Different concentrations of LAM are successfully detected
- Successful detection of 250 pg/ml
- Sufficient Limit of Detection for real urine



Example of measurement curve

[LAM]	Δλ(nm)
250 pg/ml	0.16
500 pg/ml	0.34
1 ng/ml	0.49
50 ng/ml	0.69
1 µg/ml	4.89



Calibration curve

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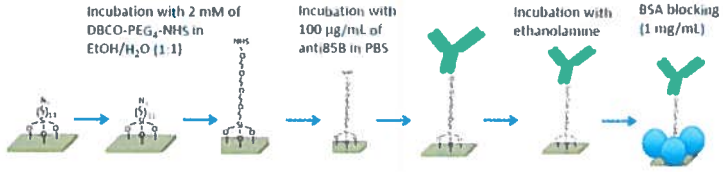
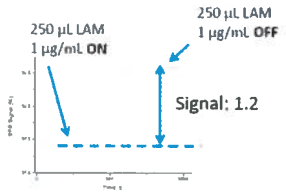
Ex-situ functionalization procedure



10 µL antiLAM
500 µg/mL
2 h incubation



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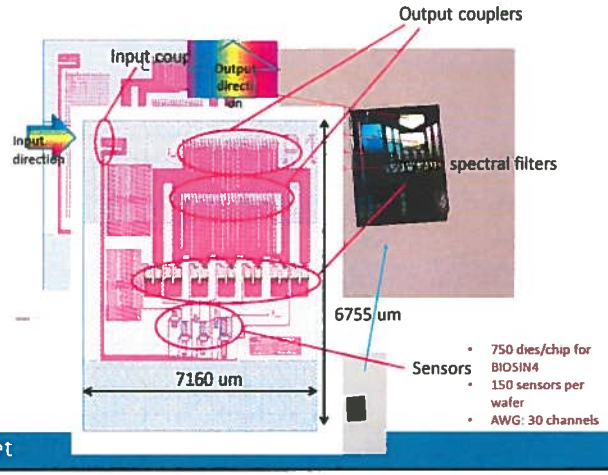
Development of low-cost point-of-care test for Tuberculosis Diagnosis



- 750 dies/chip for BIOSIN4
- 150 sensors per wafer
- AWG: 30 channels

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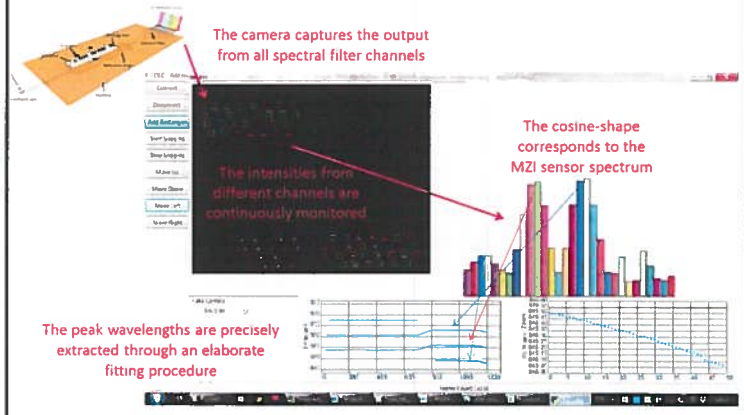
Measurement procedure



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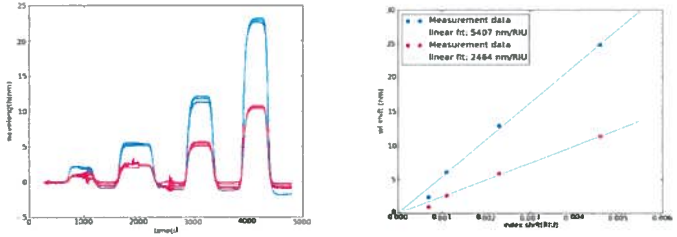
Multiplexing



- 2 different sensors measured simultaneously
- 6 sensors present on a single chip
  - Detect multiple antibodies
  - Reference sensor without receptor coating
  - Reduce error

Pocket 11

Point of care: The ultimate goal of photonic biosensors

= A device that can be taken to the patient and operated by non-specialists

Application level
- Limited/no sample preparation

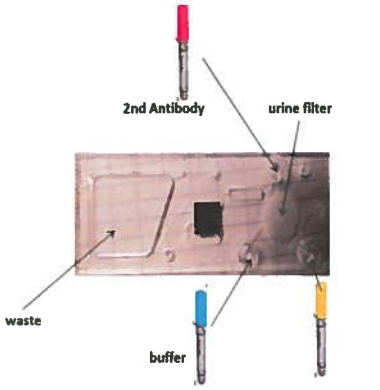
Device level
- Easy to use
- Operational under a range of conditions

Sensor level
- Cheap
  - Disposable
- Adequate limit of detection

Pocket 12

Microfluidic chip as transfer medium

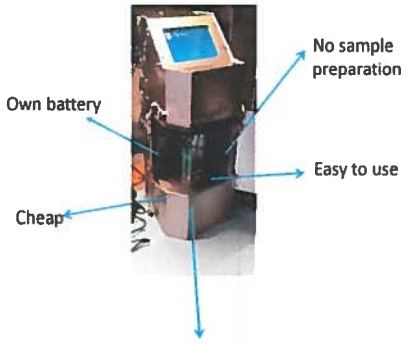
Integrate the silicon nitride chips directly in a polymer fluidic cartridge, while leaving parts of the chip exposed for easy optical access



ChipShop

Pocket 13

POCKET POCT



trinean

Pocket 14

Development of low-cost point-of-care test for Tuberculosis Diagnosis

Photonic transducer: MZI
Ghent University, Belgium

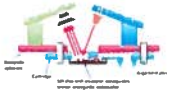
Biofunctionalisation and bioanalytical application
ICN2, Barcelona

Antigen and antibodies for the detection TB (TB biomarker)
LIONEX, Germany

POCKET POCT

Silanisation and 2nd generation of devices
Imec, Germany

Integration
TRINEAN, Germany



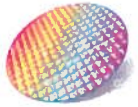
Microfluidics
MFCS, Germany

Real Samples evaluation in Africa and Asia

Pocket 15

Towards an industrial process

Funtionalisation at wafer level



Spotting of the antibodies

Implementation of capillary pumps

Packaging of the biochip

Pocket 16

Conclusion and outlook

- Point-of-care photonic sensor with bulk limit of detection of $7 * 10^{-7}$ RIU and potential for multiplexing
- Successful detection of 250 pg/ml LAM, a biomarker for TB found in urine
- Field tests planned in 2017

Thanks

Abstracts CPOCT Symposium

AACC CPOCT INTERNATIONAL SYMPOSIUM

The Benefits and Challenges of Point-of-Care Testing Across the Clinical Spectrum
September 21-24, 2016, Copenhagen, Denmark
Accepted Abstracts

Poster Author/Title **Submitting**

SESSION 1: POCT IN THE INTENSIVE CARE SETTING

P1 Allon Reiter: Diagnosis of Infection Utilizing Accellix CD64

P2 Paloma Oliver: Precision and agreement of 266 strip-based glucose meters without the involvement of the laboratory medicine

P3 Hanneke Buter: Plasma glutamine levels before cardiac surgery are related to post-surgery infections; an observational study.

P4 Nuha Al Humaidan: Analytical Performance of Point of Care Blood Gas Analyzers In The Operating Theaters

P5 Zachary O'Brien: Novel POC Devices for Testing Procalcitonin (PCT) in ER and ICU Settings

P6 James Nichols: GEM Premier 5000 Clinical Evaluation

P7 Gareth Davies: Development of an External Quality Assessment Scheme for POCT Creatinine Whole Blood Meters

P8 Alex Mewis: Organization of an external Quality Control Program for ACT Medtronic

P9 Paloma Oliver: Differences in blood gas results between POCT Neonatal Intensive Care Unit and Emergency laboratory

P31 Jin Xu Assessment of the performance of Blood Glucose Monitoring Systems for monitoring dysglycaemia in neonatal patients

SESSION 3: POCT IN THE PRIMARY CARE SETTING

P32 Eunhee Nah Clinical Value of the Urinary Albumin-to-Creatinine Ratio Measured Using a Strip Test in Prediabetes and diabetes

P33 Kirsi Luttinen-Maunu Nurse-managed Anticoagulation Clinic in Finland--How Point-of-care testing (POCT) affects TTR in Primary Care Setting

P34 Maurice Laville Creatinine level in capillary blood, a new tool for instant estimation of glomerular filtration rate at home or in ambulatory care settings

P35 Marijana Vucic Lovrencic Diagnostic performance of a point-of-care glucose analyzer in gestational diabetes

P36 Sanne van Delft Analytical performance, agreement, and user-friendliness of automated POCT urine test strip analysers, and a comparison between man and machine

P37 Kirsi Luttinen-Maunu Quality Management of Point-of-care testing (POCT) process in nurse-managed anticoagulation clinics

P38 Ayssar Elamin Tuberculosis POCT: An Integrated Photonic Biosensor for Tuberculosis Detection

P39 Lara Harmans Implementation of quality assured POC testing in Dutch general practice

P40 Hans van Pelt Evaluation of three POCT Hematology analyzers for white blood cell analysis

P41 Celine van Lint Self-monitoring creatinine after kidney transplantation: adherence to measurement protocol and reliability of patient reported data

P42 Anjly Jain Point of care creatinine testing in screening and monitoring of chronic kidney disease

P43 Javier Segarra New Emergency departments in primary care, efficiency when enhancing the role of POCT

Tuberculosis POCT: A Potential Application of Integrated Photonic Biosensor for Tuberculosis Detection

A. A. Elamin[1], D. Martens[2,3], A. B. González-Guerrero[4], M. Stehr[1], F. Jonas[1], W. Van Roy[5], R. Vos[5], A. Stassen[5], S. Severi[5], R. Bockstaele[6], P. Soetaert[6], B. Anton[7], H. Becker[7], L. M. Lechuga[4], P. Bienstman[2,3], M. Singh[1]

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[7]*microfluidic ChipShop GmbH, Stockholmer Str. 20, 07747 Jena, Germany*

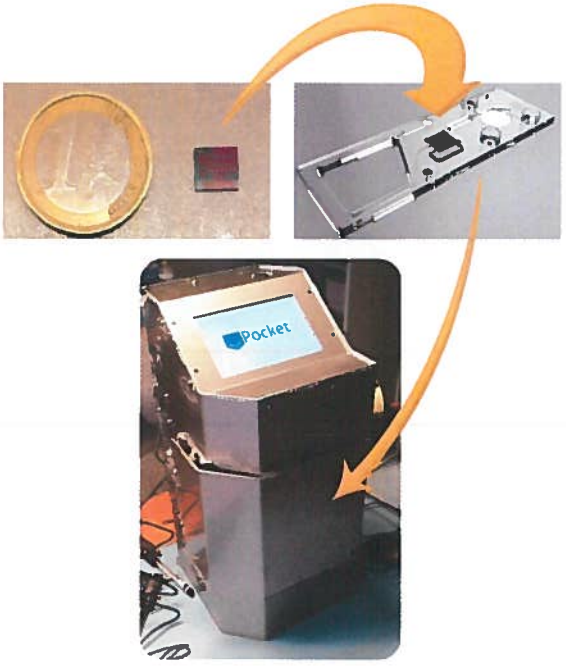
Tuberculosis (TB) is an old but re-emerging global health threat caused by the *Mycobacterium tuberculosis* (Mtb). One third of the world's population is infected with Mtb and new infections occur at a rate of one per second. Despite greatest global health impact of TB, case detection rates are low, posing serious hurdles for TB control. Current methods for the detection of TB are either time consuming or require expensive instruments. Furthermore, these tests have several limitations and perform poorly in populations affected by the HIV epidemic, are thus are not suitable for point-of-care diagnosis. Therefore, an accurate, novel, rapid, more sensitive and cost effective diagnostics are urgently needed.

In this respect, the grand goal of the Pocket project is to establish a framework to combine several state-of-the-art concepts for the development of novel and cost-effective point-of-care test for tuberculosis using patients' urine as non-invasive samples. The new tuberculosis POCT consists of a small photonic chip combined with a microfluidic cartridge (disposable part) and a graphical user interface instrument, used for optical readout and data processing (Figure 1). An integrated label-free photonic circuit is used as biosensor, a low-cost mechanism due to its small size and the compatibility with mature CMOS fabrication technology. The sensing circuit is implemented, combining a highly sensitive Mach-Zehnder interferometer with an on-chip spectral filter, hence replacing the conventional tunable laser by a much cheaper broadband light source. Flood illumination on the input grating couplers was used to reduce the cost and increasing POCT compatibility. The successful development of a POCT TB test depends on an Mtb-specific biomarker. Special focus set to the most promising markers; cell wall lipopolysaccharide lipoarabinomannan (LAM) and Ag85 complex. A novel, high-quality and selective antibodies were developed against Mtb LAM and Ag85 complex biomarkers. This unique cocktail promises to significantly enhance the sensitivity and specificity far beyond current TB tests. In a preliminary experiment, sensor chips were functionalised using an Azide-ended silane by vapor phase deposition and antibodies were bio-conjugated by click-chemistry using a PEG-based linker. Initial results indicate the successful detection of 250 pg/ml of LAM antigen, thus demonstrating its potential for use in resource-limited area and for the on-line diagnosis of TB. In the new POCT, the safety of sample process has been successfully implemented using microfluidic chip as transfer medium. The designed chip has very low fabrication costs, allowing cost-effective disposable chips to be fabricated in mass production. This chip is plugged into the measurement tool, which contains the required components for optical readouts, an automated system to circulate the urine into the chip as well as a computer for data processing.

Due to rising health-care costs, all health-care stakeholders are forced to shift their onus from a 'pay for intervention' to a 'pay for performance' model. The highly promising TB POCT need to be evaluated in order to determine a universal threshold, especially in endemic countries as well as performance in the field. Hence, there is a need and justified rationale for performing medium/large evaluation trials which will be our near future step.

Acknowledgement: The Pocket project was funded by the European Commission under grant agreement no FP7 610389.

Attachment: Figure 1: TB POCT Instrument and the disposable chip parts



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