DYSSEE

A modular platform for field diagnosis of Tuberculosis.

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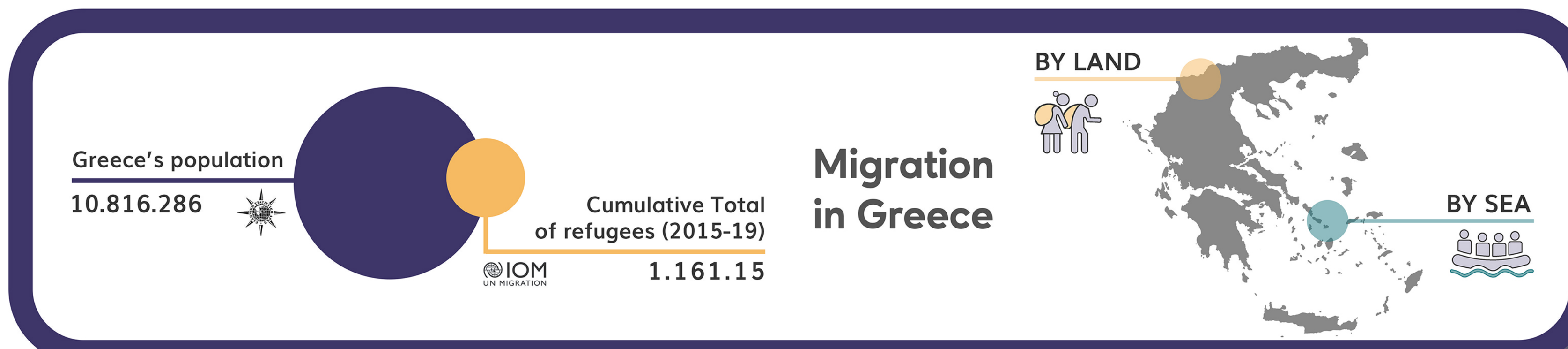


01. OVERVIEW.

TB *Mycobacterium tuberculosis*

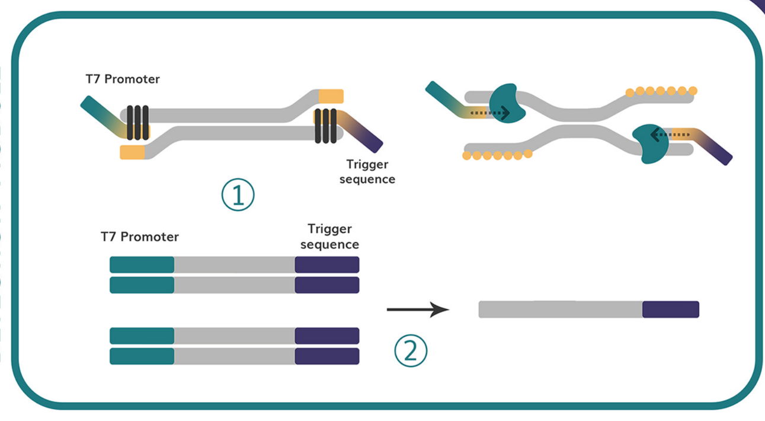
1 of 10 causes of death worldwide

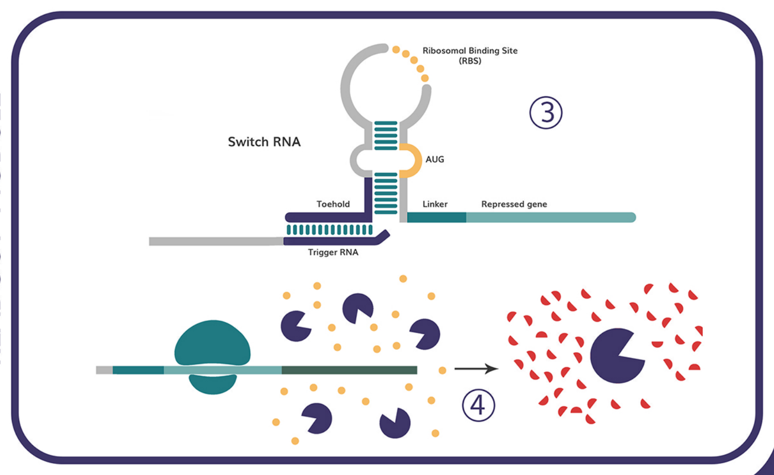
10m patients
1.3m deaths

3.6m people underdiagnosed

World Health Organization Global Tuberculosis Report 2018

Aiming to tackle the TB epidemic, we developed a two-module diagnostic test that detects fragments of the Mycobacterium-specific gene, IS6110, in patient's urine [1] and produces a colorimetric readout. In our system, four steps of signal amplification, including: isothermal amplification, *in vitro* transcription and translation are used in a biomarker detection module. During this detection step, an oligonucleotide trigger is incorporated in the amplified biomarker. This trigger links the detected biomarker with the second readout module by acting as a translation activation switch driving expression of b-lactamase, which in turn produces a colorimetric readout. Results of our detection system can be visualized by naked eye. We envision incorporating these two modules in a field test. Our design can be easily implemented for detection of several diseases due to the universality of the detection and readout modules.





INSPIRATION.

People on the move have many problems to face. During their journey to our country, Greece, they become vulnerable to infectious diseases due to limited access to healthcare. The most frequently screened disease among newly arrived migrants and refugees is Tuberculosis [2]. For this reason, our test is initially destined for deployment in migrant refugee camps in Greece. We aim for its future use worldwide as a first step towards achieving universal health care.

02. MODEL.

- Functional for the cell free system
- Calibrated according to experimental data
- Predicted a robust signal at 30-40min

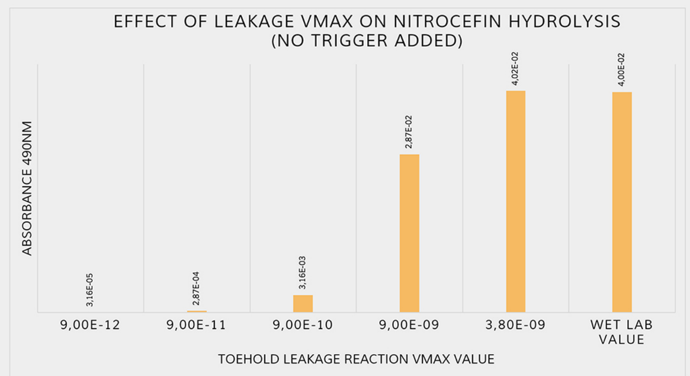


Figure 1. Calculated absorbance of hydrolyzed nitrocefin in different Vmax values for the leakage reaction (no trigger added).

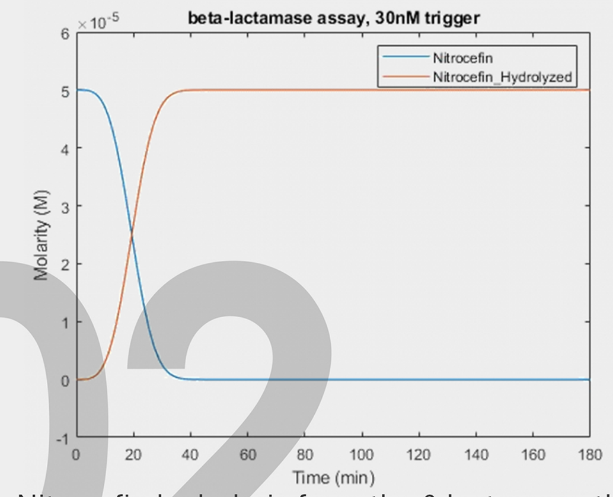


Figure 2. Nitrocefin hydrolysis from the β-lactamase through the *in vitro* transcription and translation system leading to the prediction of the minimum time that is needed for the reaction to be complete.

03. PARTS.

- Incorporation of the BioBrick characterization and improvement into our project design

Improvement (BBa_K2973007)

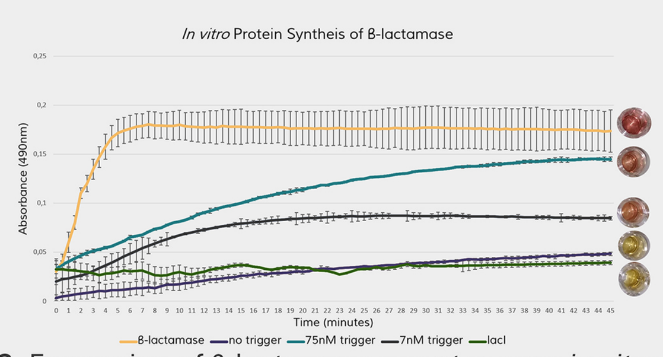


Figure 3. Expression of β-lactamase reporter gene *in vitro*. Error bars represent the standard deviation for n=2 technical replications.

Characterization (BBa_I757010)

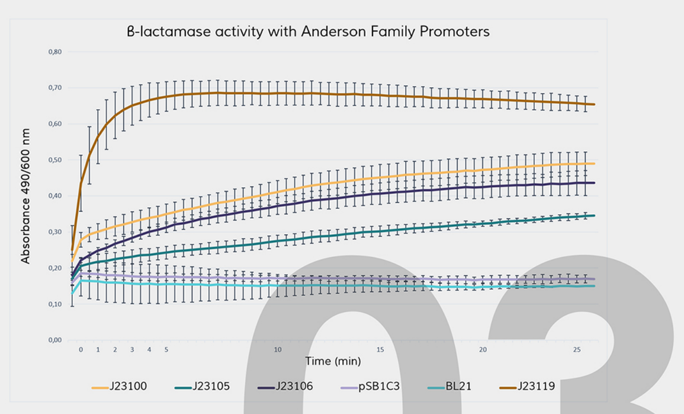


Figure 4. The hydrolysis of nitrocefin enabled by the expression of the β-lactamase gene, under the control of different promoters (J23100, J23105, J23106 & J23119) of the Anderson family. The substrate (nitrocefin) hydrolysis (490nm) is divided by cell growth (600nm), in order to normalize all values.

04. RESULTS.

Detection Module

- Incorporates an isothermal amplification method (RPA)
- Optimal working conditions: 5min | 42°C
- Functions successfully in DNA fragments
- Adjusted primers and conserved universal overhangs leading to the detection of any DNA sequence
- Works for Hepatitis B virus detection

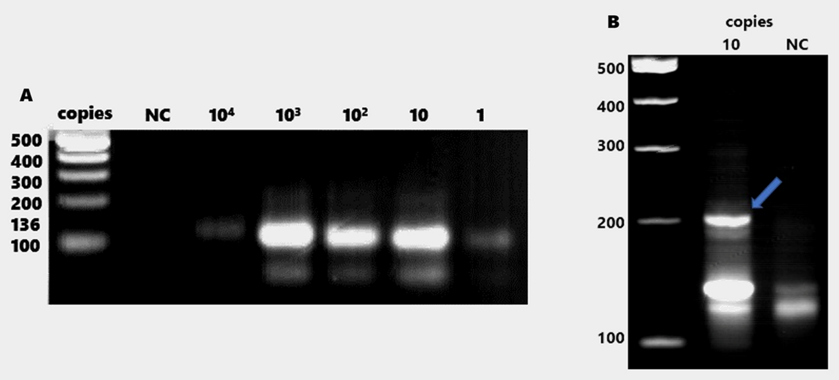


Figure 5. A) Detection of the IS6110 biomarker through Recombinase Polymerase Amplification (RPA). B) Detection of an HBV genome fragment using RPA.

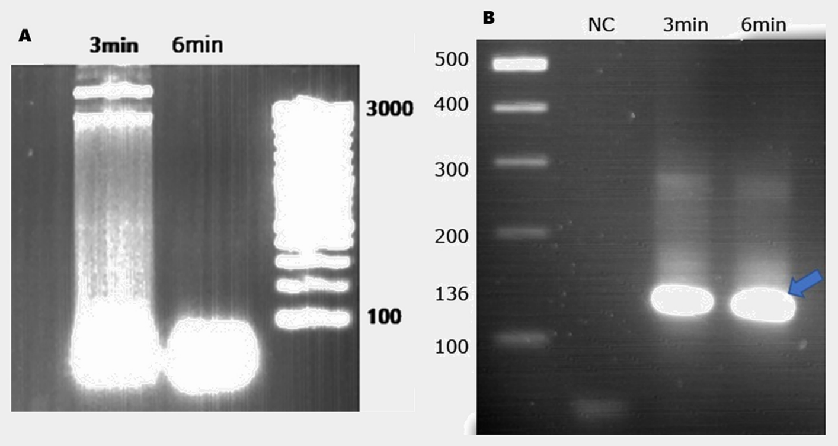


Figure 6. A) Random fragmentation of the MTB IS6110 biomarker using DNaseI non-specific endonuclease. B) Amplification of the desired part of the DNaseI treated biomarker using RPA.

Readout Module

- Uses amplified sequence as a trigger (input)
- Trigger derives from the detection module
- Incorporates newly designed toehold switch and trigger

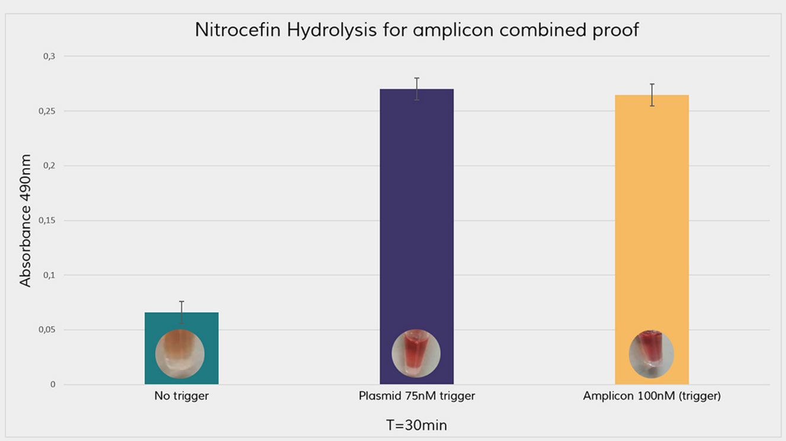


Figure 7. Measurement of toehold-regulated β-lactamase when the trigger is the previous step's amplified sequence or in a plasmid vector.

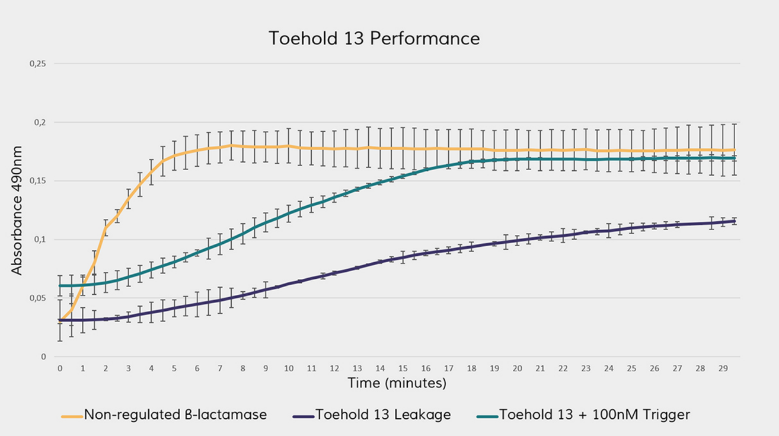
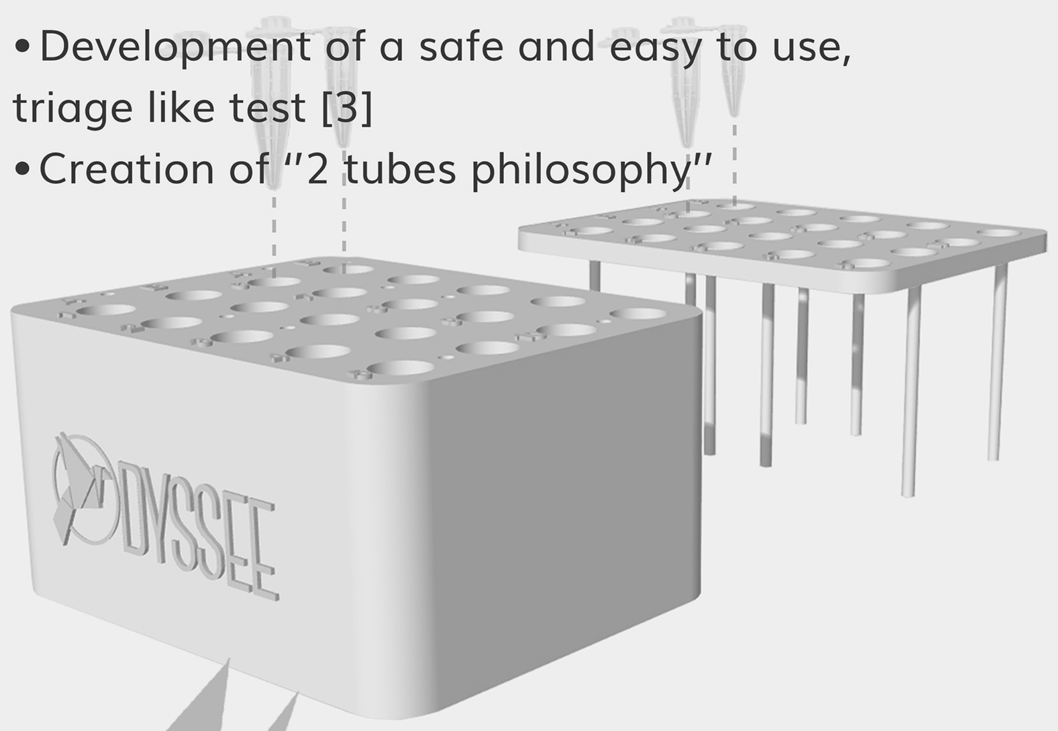


Figure 8. Performance of toehold 13 in a β-lactamase enzymatic assay. Sequence of toehold 13 derives from *Geobacillus kaustophilus*. Error bars represent standard deviation of n = 2 replicates. Blank was subtracted.

05. PRODUCT DESIGN.

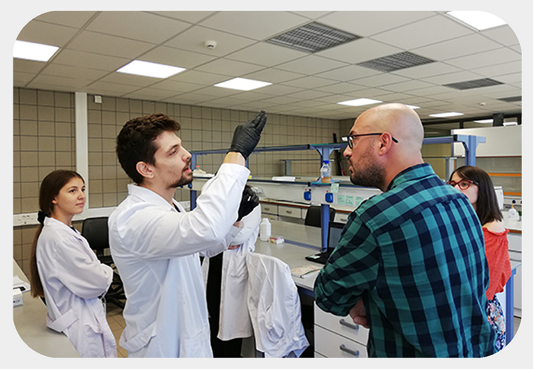
IMPLEMENTATION.

- Development of a safe and easy to use, triage like test [3]
- Creation of "2 tubes philosophy"



DEMONSTRATION.

- Deployment at the refugee facility "Agia Eleni" in Ioannina, Greece
- Confirmation of our test's simplicity by the Senior Supervisor



06. ENTREPRENEURSHIP.

Strengths	Weaknesses
-Non-invasive, use of urine as biological sample -Nospecialized personnel needed in order to be executed -Adapted especially for the screening of populations and Low Resource Settings -Time-efficient -Can be used for treatment monitoring -Universal platform for the diagnosis of several diseases -Sale for user	-Dianosis only of active TB -Technology not proven in large scale -Some reagents have an early expire date and need to be stored in fridge
Opportunities	**Threats**
-Incorporating SynBio for the diagnosis TB in the market -The TB diagnosis market is big -Future implementation in open polyclinics and hospitals -Few competitors using the same approach in urine Samples	-Incorporating SynBio for the diagnosis TB in the market -The TB diagnosis market is big -Future implementation in open polyclinics and hospitals -Few competitors using the same approach in urine Samples

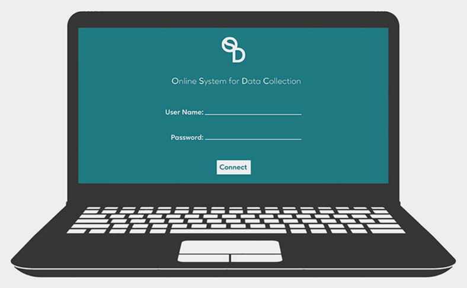
- Potential implementation in Open Polyclinics (Doctors of the World)
- Incorporation into TB programs (World Health Organization)
- Funding opportunity by the Hellenic Foundation for Research and Innovation

07. HUMAN PRACTICES.

INTEGRATED HP.

Healthcare model.

- To be communicated to policy makers
- Containing an electronic platform for data collection



Application.

- Discussion with experts from Humanitarian organizations
- Implementation in Reception and identification centers

Product Guide.

- Making our system accessible to all
- Translated into Arabic, French and Urdu

08. EDUCATION& PUBLIC ENGAGEMENT.

- Communication science to the general public, academic community, and industry
- Promotion of barrier-free education

OMIC ENGINE · IKY · Erasmus+ · NOVARTIS · ELPEN · HELLENIC PETROLEUM · TWIST BIOSCIENCE · NEW ENGLAND BioLabs · Lab Supplies Scientific · IDT · MathWorks

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1. Fernández-Carballo, B. L., Broger, T., Wyss, R., Banaei, N., & Denkinger, C. M. (2018). Journal of Clinical Microbiology, 57(4), 1–9.
2. World Health Organization. (2018). Report on the health of refugees and migrants in the WHO European Region.
3. WHO Report (2014). WHO Meeting Report, (April), 1–98.