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Laser-based fabrication of affordable paper-based point-of-care clinical diagnostics test

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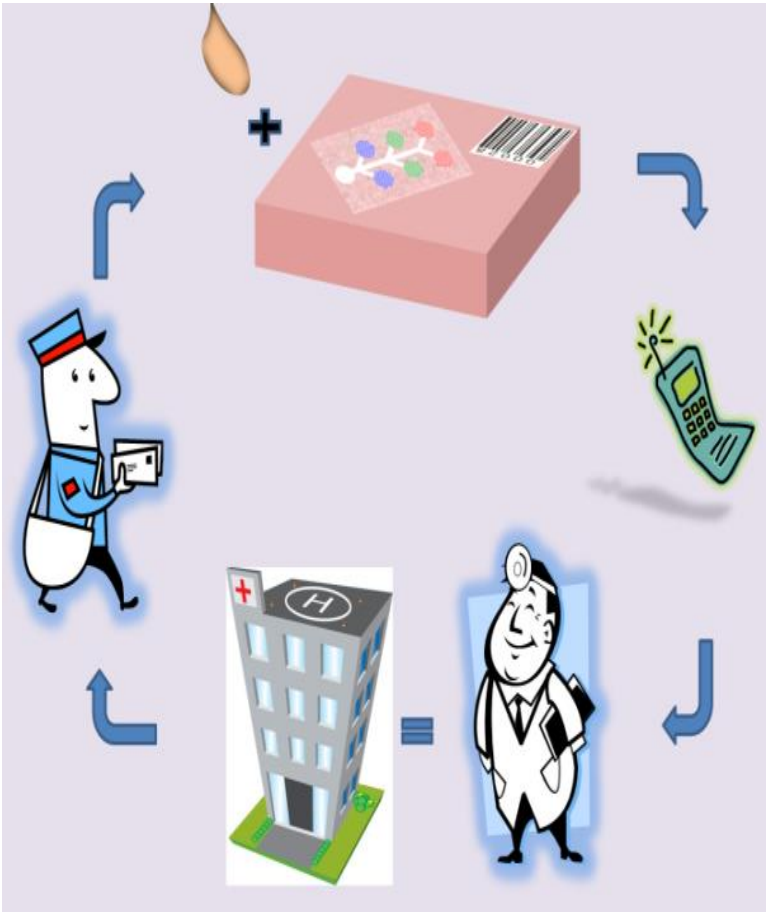
Motivation….

- Enable detection of early stage bacterial infection
  - Home-testing for infections – low cost, patient-friendly testing, rapid if possible, deliverable, readable using mobile-phones
  - As they wait to be seen by their GP or clinician post an operative surgery
- Reduce the burden of the testing
  - Free up the ‘Clinician/GP time’
- Prevent anti-microbial resistance



Paper-based fluidics

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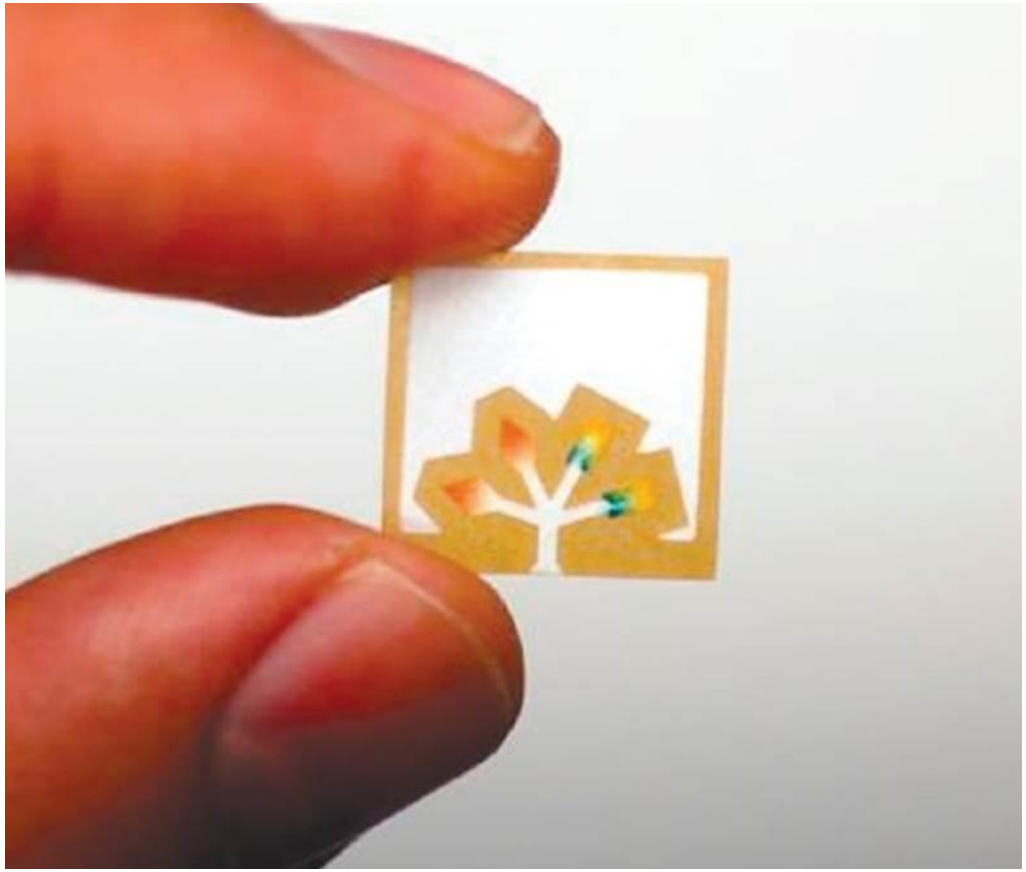
Such microfluidic lab-on-chip type devices consist of



Interconnected hydrophilic fluid-flow channels

+

Hydrophobic barrier walls that extend throughout the paper

Requirements –

- Pattern paper to form the fluidic patterns
- Deposit biological materials for implementing the assay/test

LDW patterning approach

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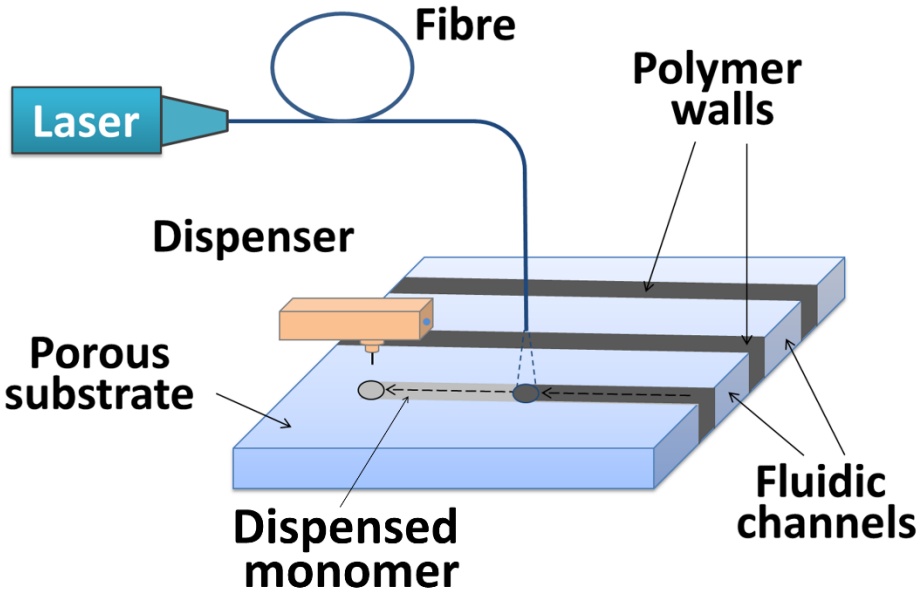
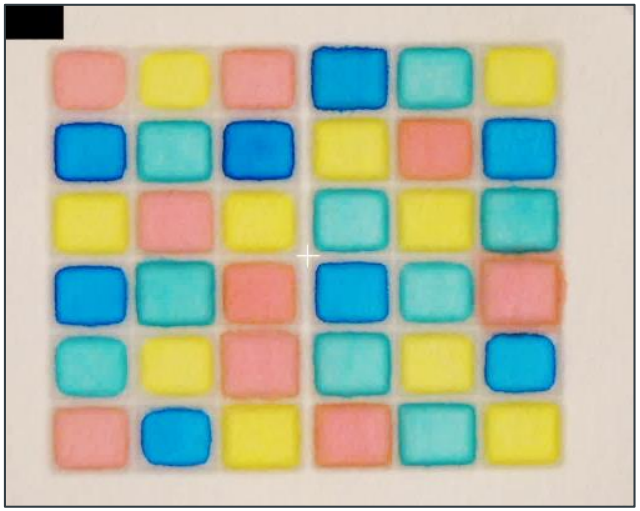
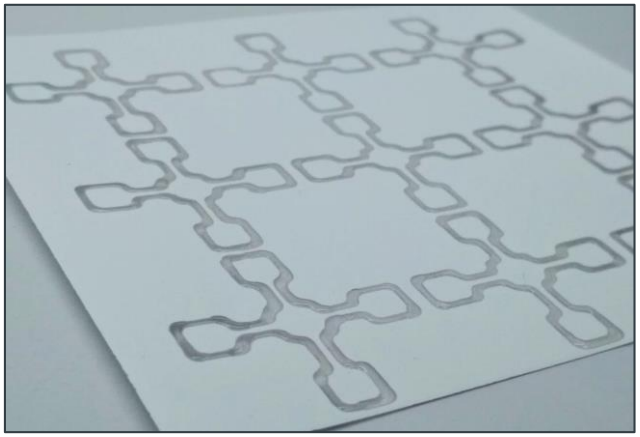
Technique that allows creation of μ-fluidic devices in porous materials

1. A local-deposition assisted laser-direct write procedure
2. Relies on the concept of light-induced polymerisation







Lasers used
Few mW of 405 nm c. w. lasers

Polymers used
Desolite 3471-3-14

Porous materials patterned
Cellulose, Nitrocellulose membranes, glass-fibre filters, and fabrics

Introduction to Lateral-flow Devices

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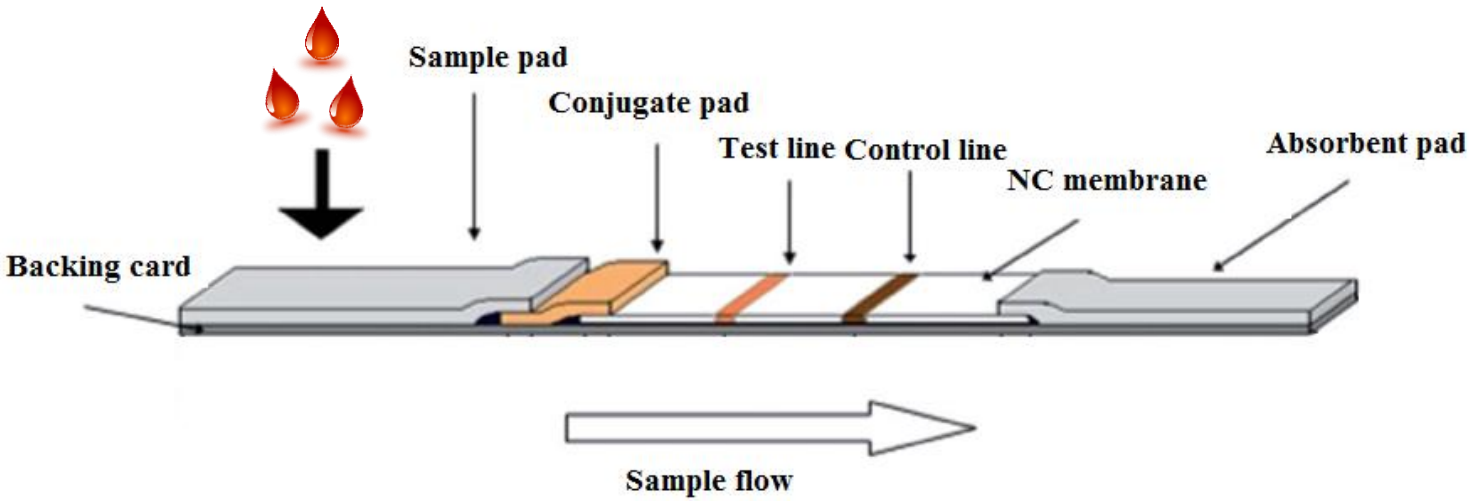
Lateral Flow Devices (LFDs) or Dip-Sticks

- Simple devices intended to detect the presence (or absence) of a target analyte in sample (matrix) without the need for specialized and costly equipment.
- A widely spread and well known application is the home pregnancy test.



Introduction to Lateral-flow Devices

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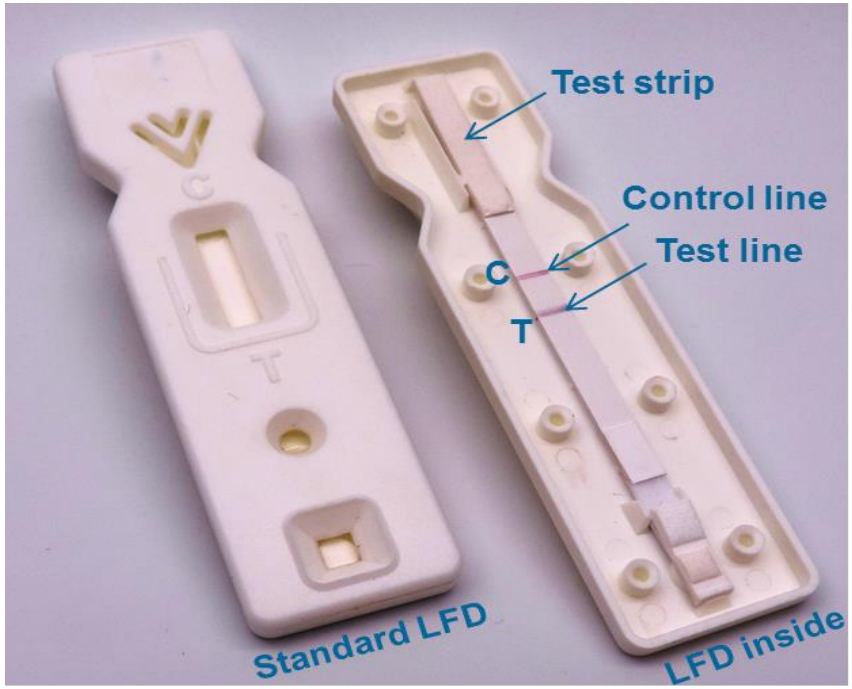
Lateral Flow Devices (LFDs) or Dip-Sticks

Their advantages -

- ✓ Used at point-of-care
- ✓ Provide rapid results (~few min.)
- ✓ Are easy-to-use
- ✓ Are affordable (~ few £s)



Their disadvantages -

- X Detect a single condition or disease only
- X Give a yes/no answer only
- X Have low sensitivities

Hence have minimal clinical use

Our solutions to Lateral-flow Devices

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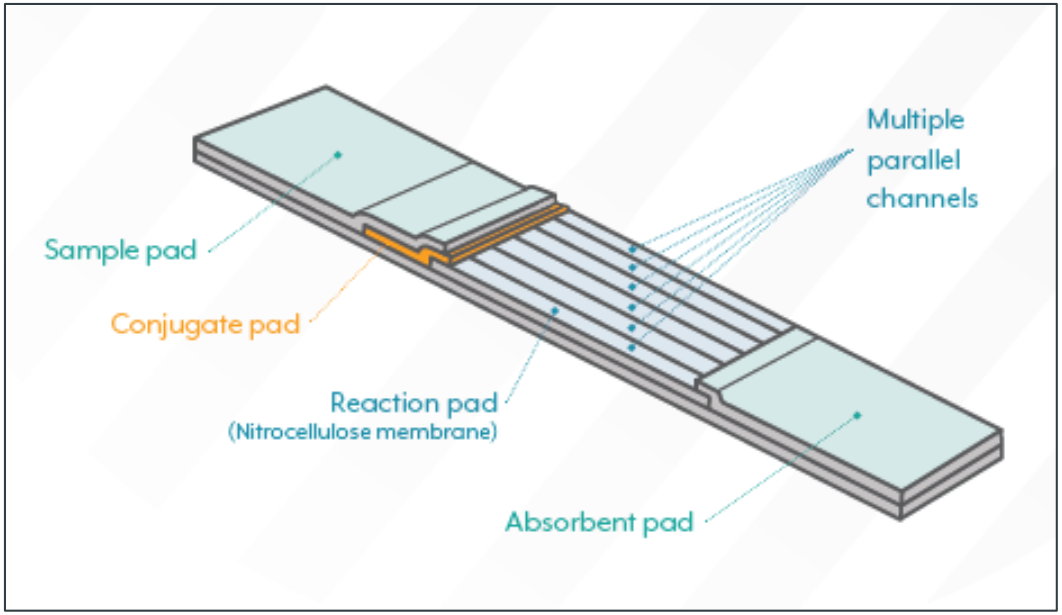
- Multiplexed detection
  - Partition the flow path of a lateral flow test into multiple parallel channels that allow detection of multiple diseases.



- In collaboration with the UHS and the Federal University of Minas Gerais, Brazil, we have developed a multiplexed LFD for diagnosis of Visceral Leishmaniasis
- In collaboration with the UHS, we are developing a multiplexed LFD for diagnosis of Tuberculosis
- In collaboration with the UHS, we are developing developed a multiplexed LFD for Dementia

Multiplexed detection on LFDs

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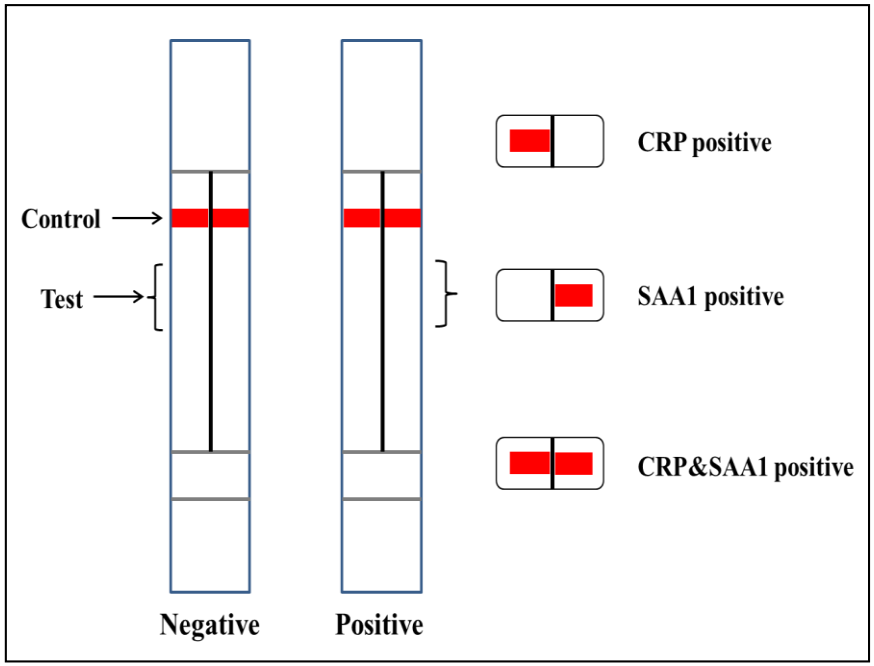
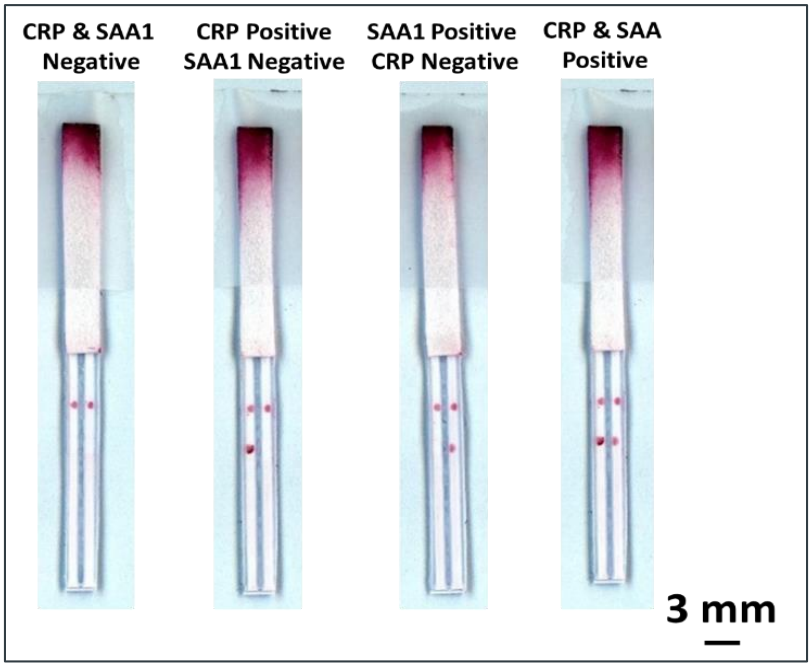
Our solution – multiplexing in multiple isolated flow-paths





Results for detection of CRP and SAA1 using LFDs with multiple flow paths.

Advantages:

- No interference of multiple test sites positioned in the same flow path
- No need for increased device dimension
- No need for addition sample volume

Our solutions to Lateral-flow Devices

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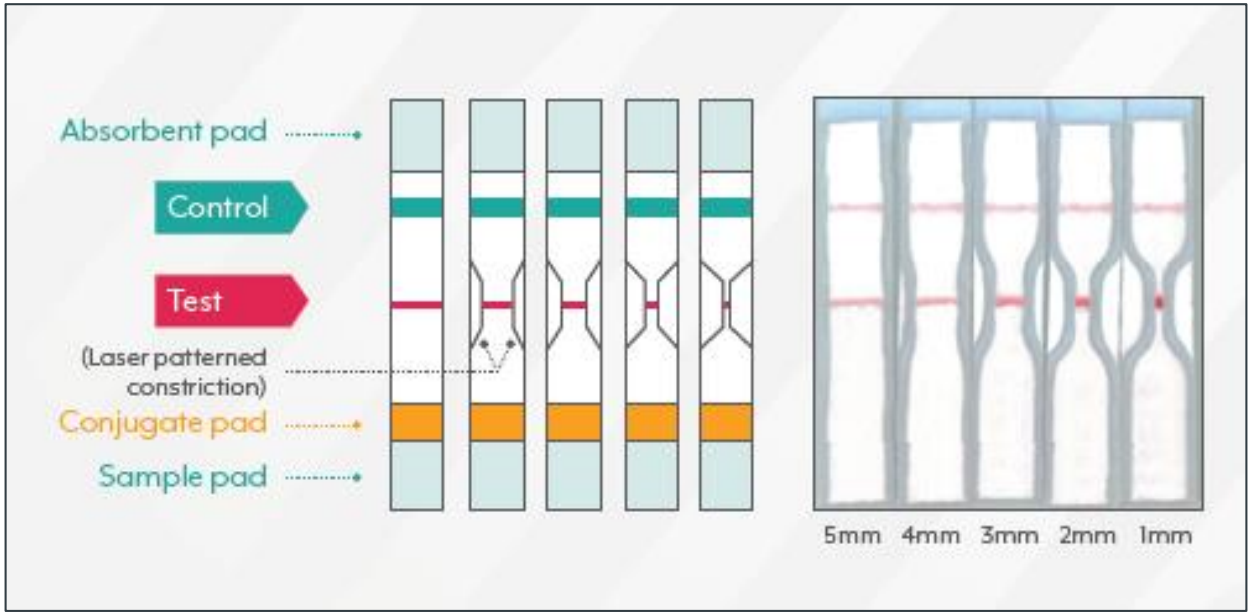
- Increased sensitivity
  - Shape the flow path of a lateral flow test to have a custom-designed constriction that produces a enhanced high sensitivity.



Improved sensitivity and limit-of-detection on LFDs

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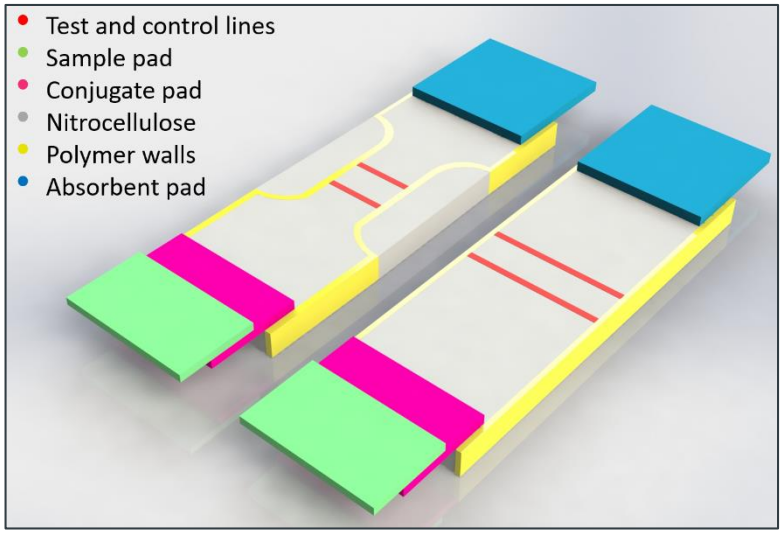
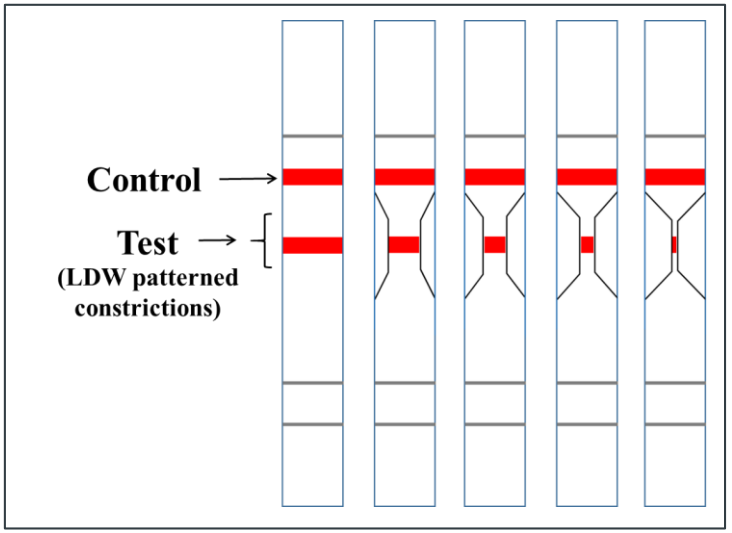
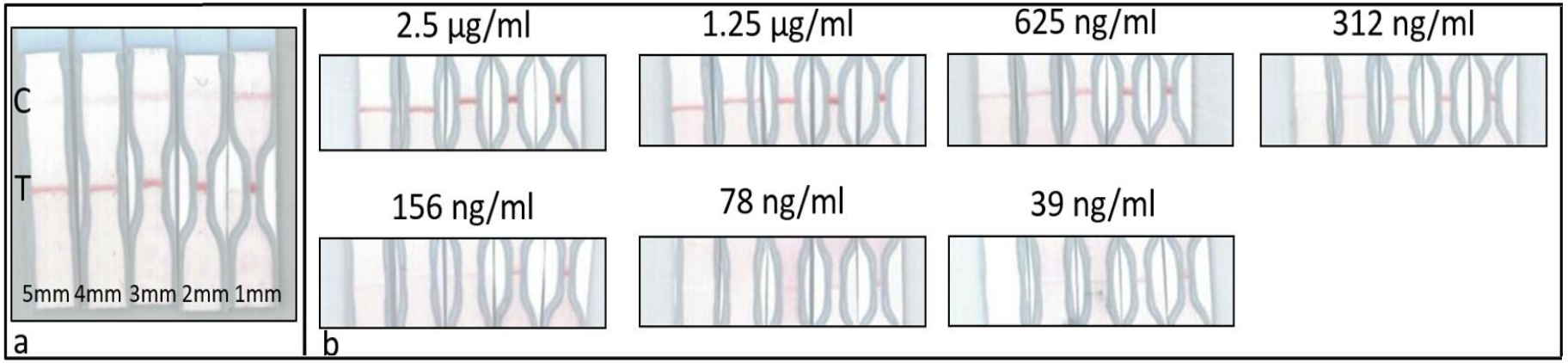
Example– sensitivity and limit-of-detection increase for a CRP assay







Our solutions to Lateral-flow Devices

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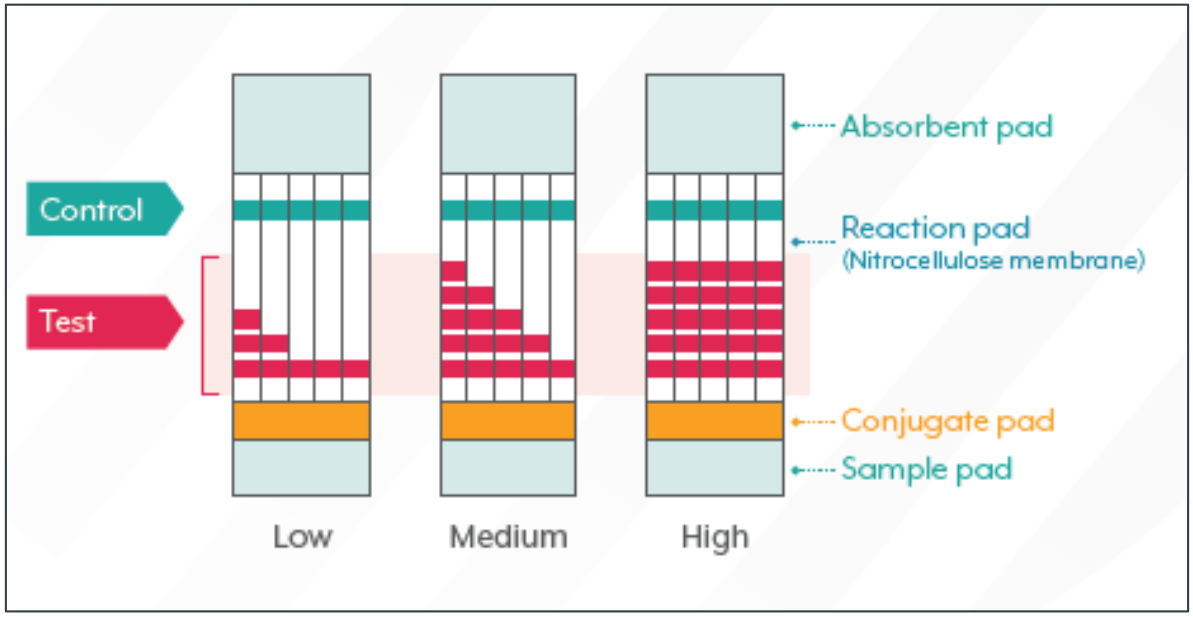
- Quantitative testing
  - Partition the lateral flow test strip into multiple channels, each of which is pre-loaded with a set number of test lines. A quantitative result will be given by the number of lines that appears.



Our Solution to AMR

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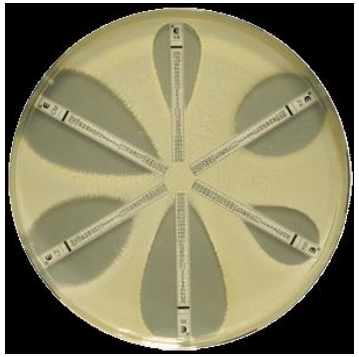
- Current approach
  - Agar-plate based bacterial culture in pathological labs
  - Results visualized by highly trained experts



- Our solution

Create the laser-patterned devices – in the desired porous substrate

A. Impregnate the pre-structured paper with chromogenic agar/nutrients
   1. Different 'Defined Media' in individual wells to allow 'Permissive Growth
   2. Inoculate, incubate and then study the growth of the bacteria

B. Impregnate the pre-structured paper with nutrients and/or agar and add different antibiotics to each test zone

Integrated paper device -

for Antimicrobial resistance testing

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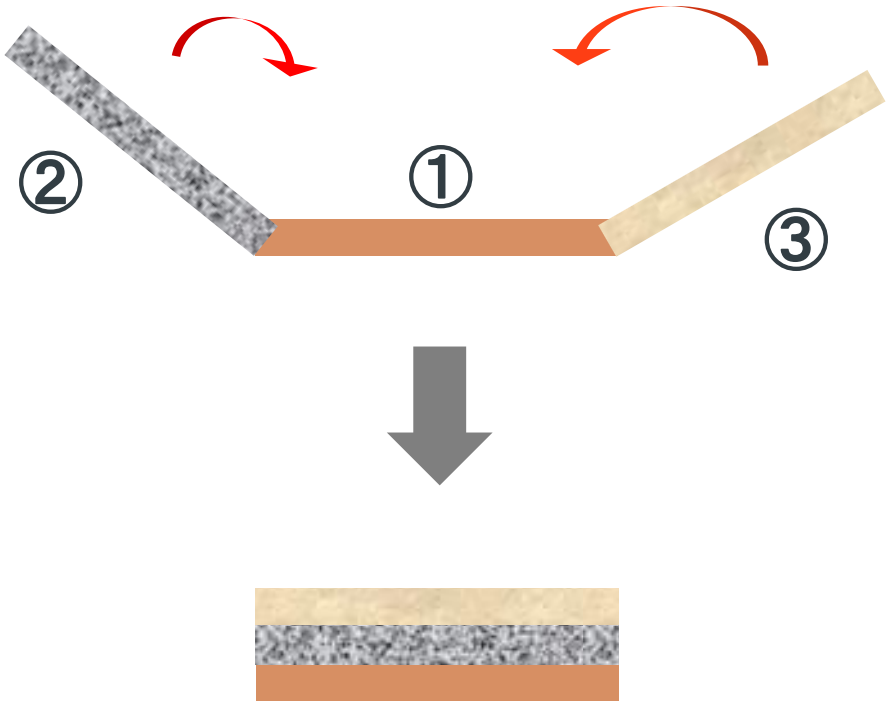
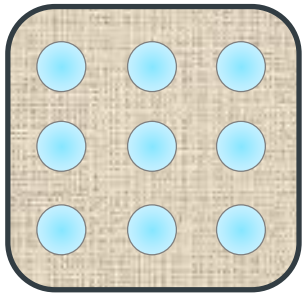
Three-layer foldable paper device enables bacteria culture and further antimicrobial resistance testing

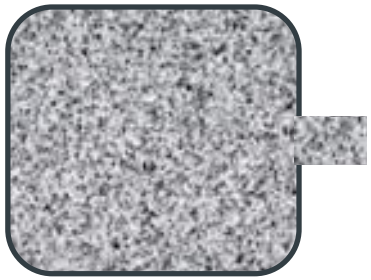


③ Antibiotics array



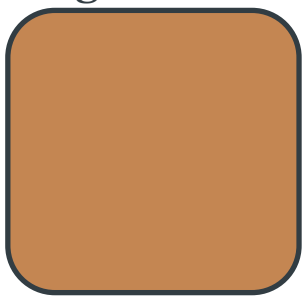
Array of individual test zones for susceptibility test

② Sample inlet



Sample pad with an inlet point extend out of the device

① Agar base



Bottom layer contains growth medium for bacteria culturing.

Integrated paper device -

for Antimicrobial resistance testing

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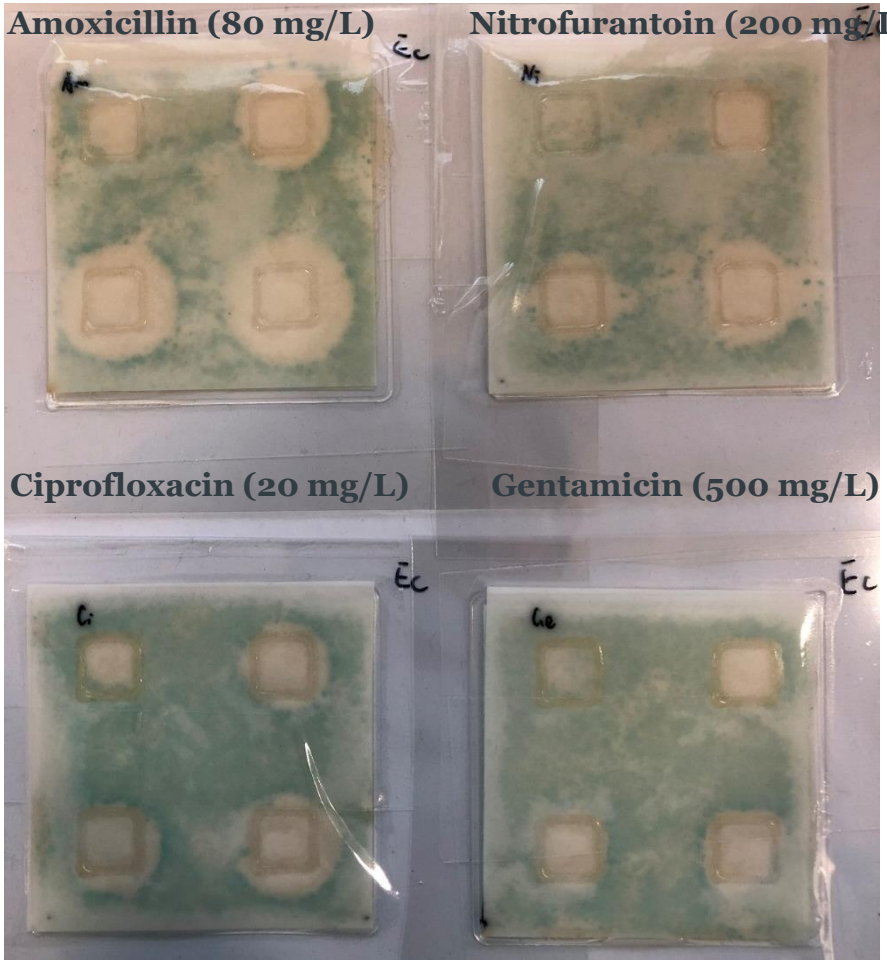
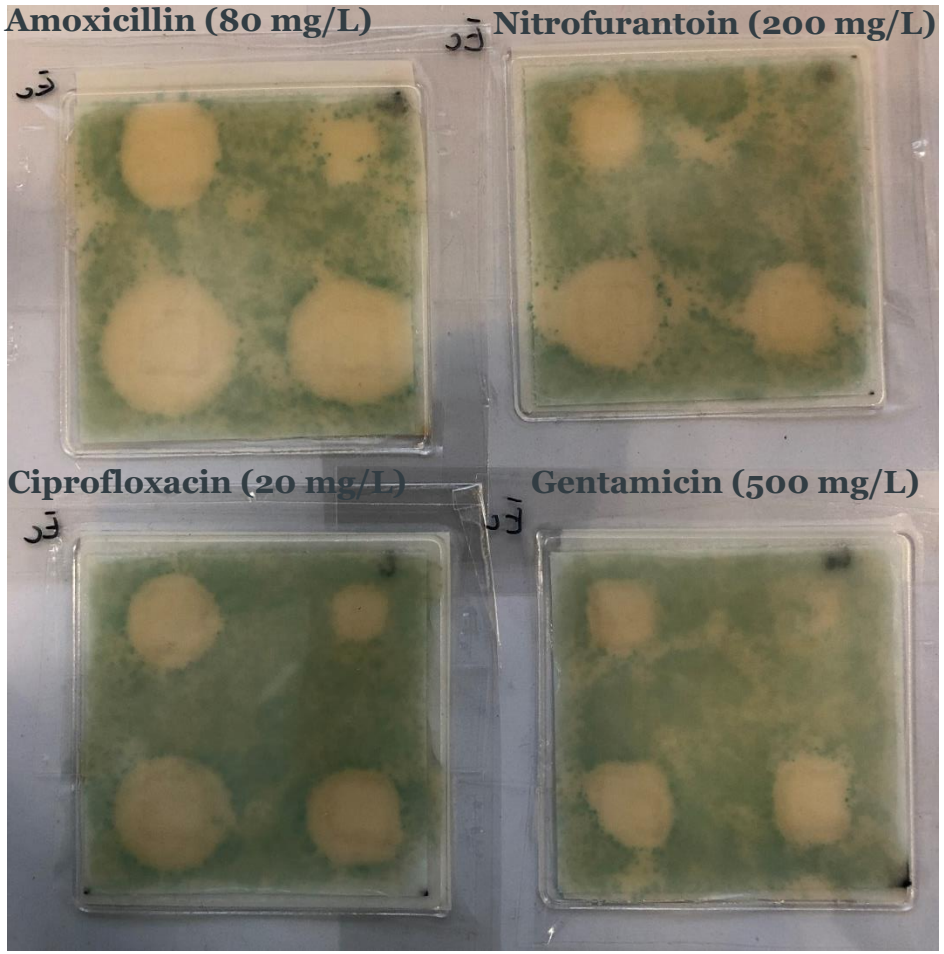
Top



Bottom



E.coli

5	15
25	35µl

Volume of antibiotic in each well

15	5
35	25µl

Normalization of paper device to conventional disk diffusion test

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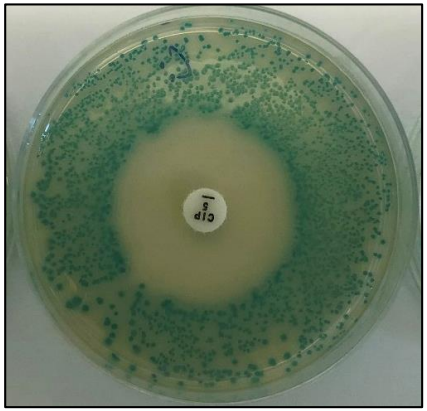
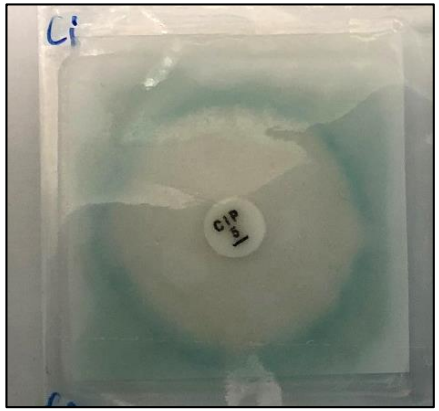
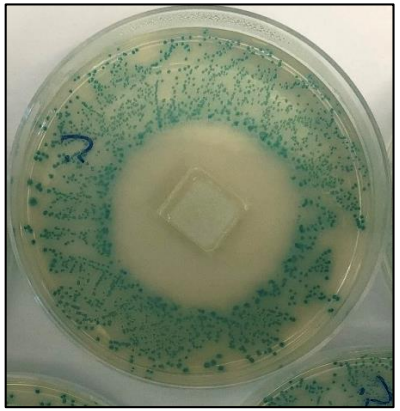
Disc on Agar



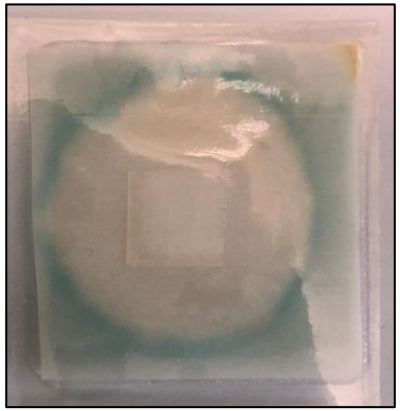
Disc on paper device



Paper disc on Agar



Paper disc on paper device



Ciprofloxacin	Avg. Dia. (mm) n=5
Disc on Agar plate	28.3
Disc on paper device	25.3
Paper square on Agar plate	28.8
Paper square on Agar-impregnated paper	25.4

* Zone diameter breakpoint (mm): S>26 R<24

Conclusion:

- Our current strain is sensitive to ciprofloxacin
- Antibiotic susceptibility testing on paper-based devices produce results similar to conventional agar plate testing

Normalization of paper device to conventional disk diffusion test

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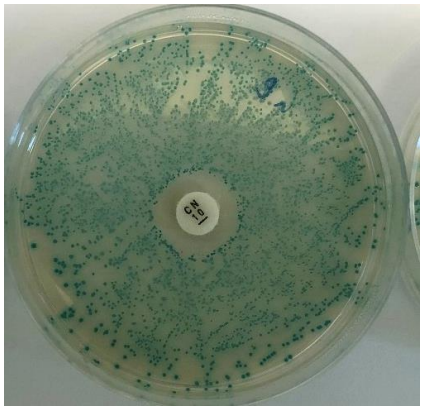
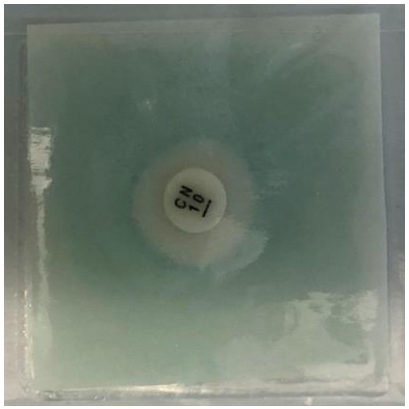
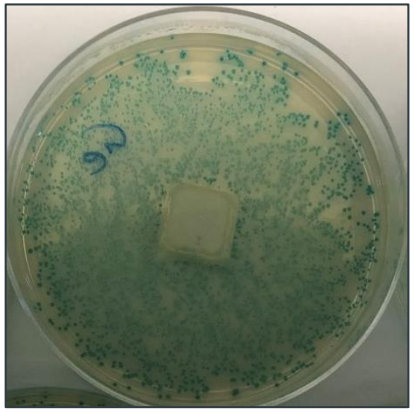
Disc on Agar



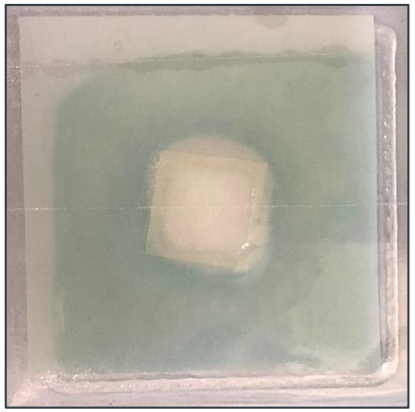
Disc on paper device



Paper disc on Agar



Paper disc on paper device



Gentamicin	Avg. Dia. (mm) n=5
Disc on Agar plate	11.3
Disc on paper device	11.7
Paper square on Agar plate	11
Paper square on Agar-impregnated paper	11

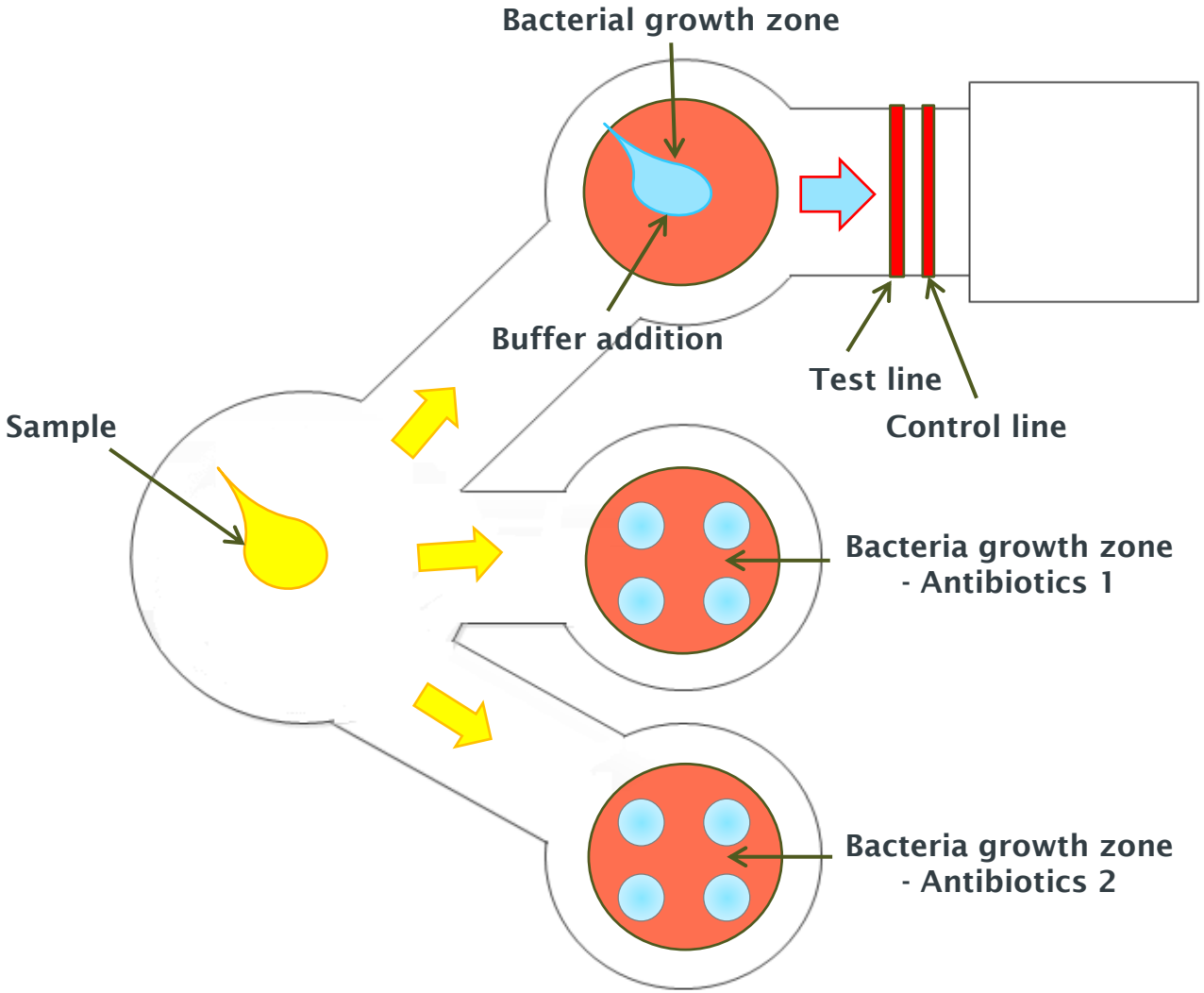
* Zone diameter breakpoint (mm): S>17 R<14

Conclusion:

- Our current strain is resistant to Gentamicin
- Antibiotic susceptibility testing on paper-based devices produce results similar to conventional agar plate testing

Final goal – in future

- Bacterial culture for antimicrobial resistance and lateral-flow test for bacteria detection on the same device



Thank you!

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Faculty of Medicine, University of Southampton

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University of Southampton
NAMRIP Pump Priming Award
Fellowship - Institute for Life Sciences and the Faculty of Medicine

Project partners

CORIS BioConcept

operon immuno & molecular diagnostics

dstl

Public Health England

AHLSTROM

MOLOGIC BIG IN TINY SCIENCE

So far

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- Successfully infused the pre-structured paper with nutrient impregnated agar and allows for E.coli bacteria growth
- Identified the best chromogenic agar for E.coli growth and identification on paper devices
- Successfully preformed antimicrobial resistant tests for E.coli with 4 commonly used antibiotics on paper devices
- Demonstration of an early-stage integrated device for E.coli culture and antimicrobial resistant tests
- Comparing and normalization of antimicrobial resistance testing on paper devices with conventional disk diffusion test

Next step

- Design and performance optimisations of the early-stage device for E.coli culture and antimicrobial resistant tests
- Testing of clinical samples
- Further validation of our paper-based device with other bacteria, i.e. Pseudomonas.
- Building of lateral-flow tests for bacteria detection on the same device

Final goal

- Bacterial culture for antimicrobial resistance and lateral-flow test for bacteria detection on the same device

Bacteria culture on paper device

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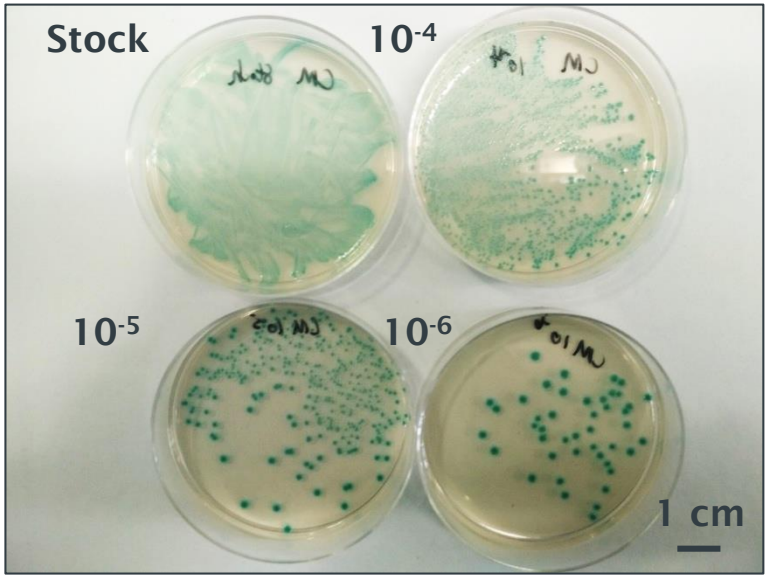
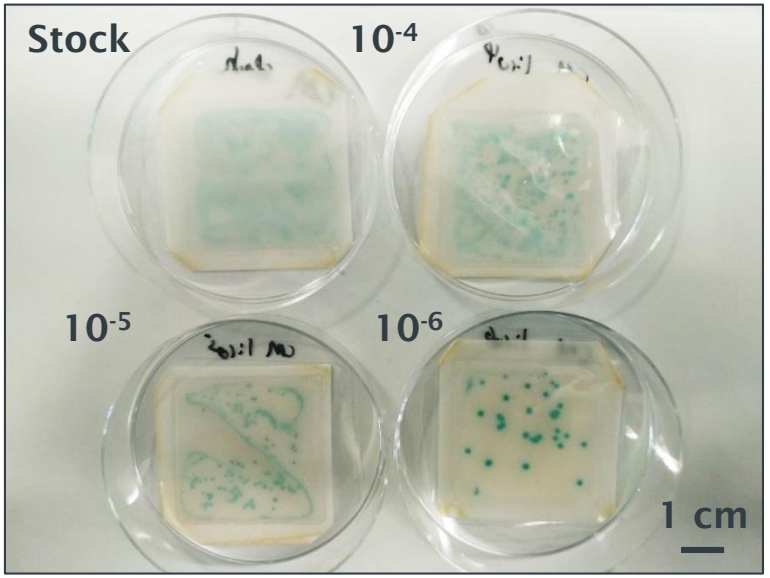
Comparison of E.coli growth: petri dish vs. paper-device





CFU count x Volume of bacterial solution (10 µL) x Dilution factor (10^{-6})

- Average count (n=3) for agar plate: 27; for paper device: 27
  CFU=2.7×10^9 CFU/mL

Conclusion:

Our paper-based devices perform identically with conventional agar plate

Antimicrobial resistance testing

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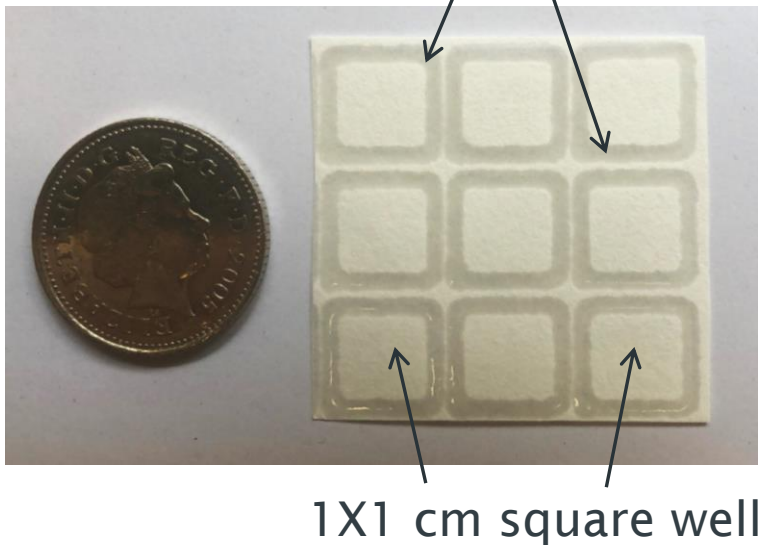
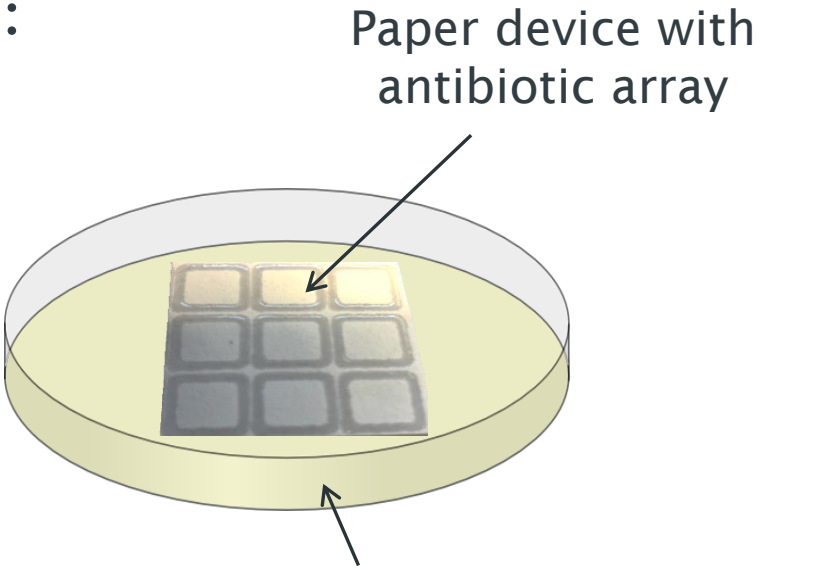
- Target pathogens – E.coli
- 3x3 array test zones (antibiotics):





Four different antibiotics have been tested at different doses

- Amoxicillin
- Nitrofurantoin
- Ciprofloxacin
- Gentamicin

Antimicrobial resistance testing

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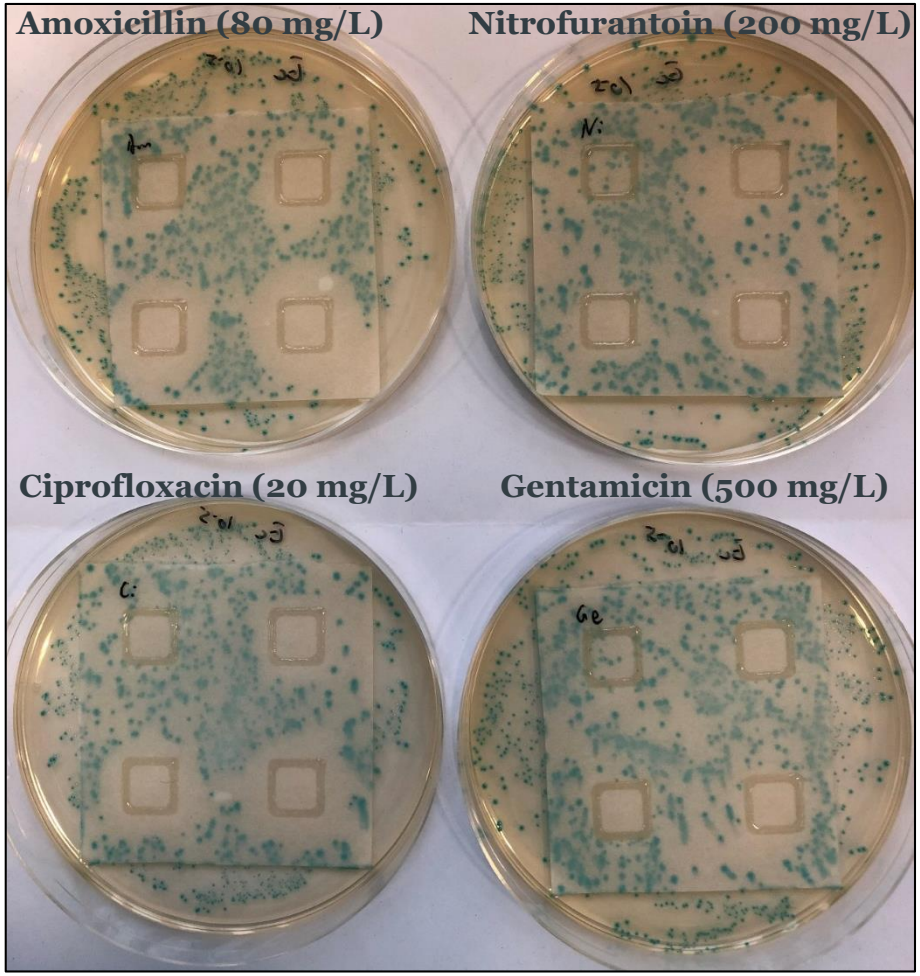
Top



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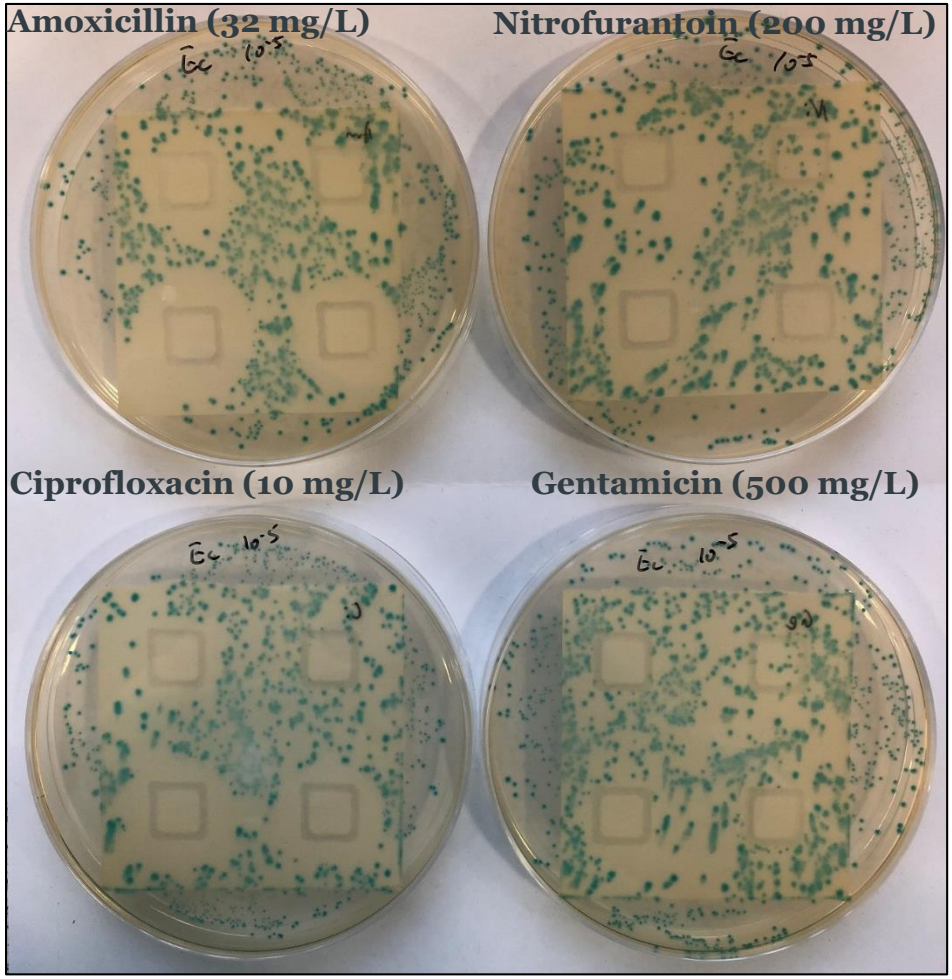

E.coli

5	15
25	35µl

Volume of antibiotic in each well

15	5
35	25µl

Bacteria culture on paper device

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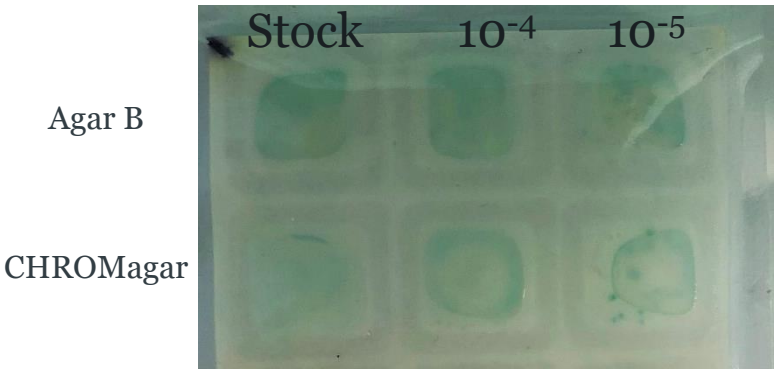
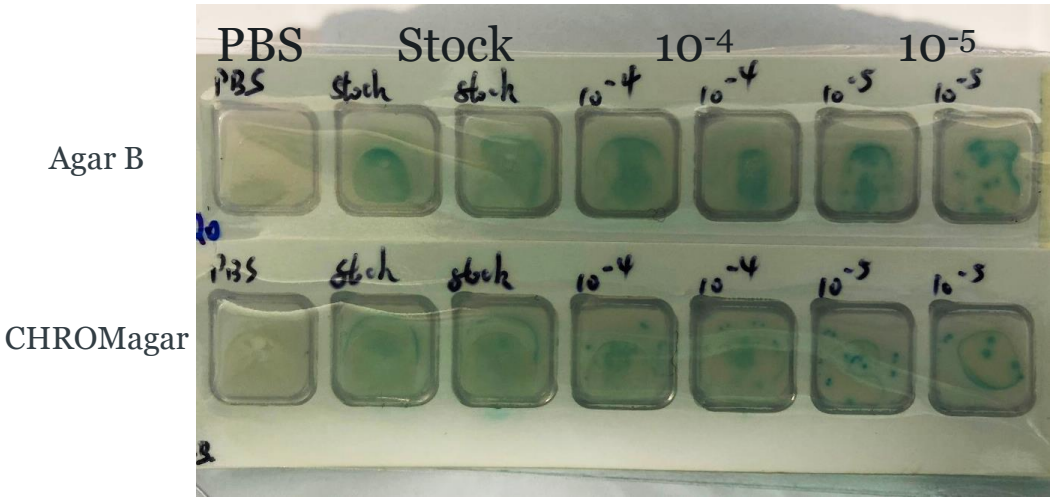
Study the growth of the bacteria

- Identify the best substrate and chromogenic agar for E.coli culturing

Cellulose filter



Nitrocellulose membrane



Chromogenic used

- E.coli ChromoSelect Agar B
- CHROMagar E.coli

Substrate used

- Cellulose filter
- Nitrocellulose membrane

Multiplexed detection on LFDs

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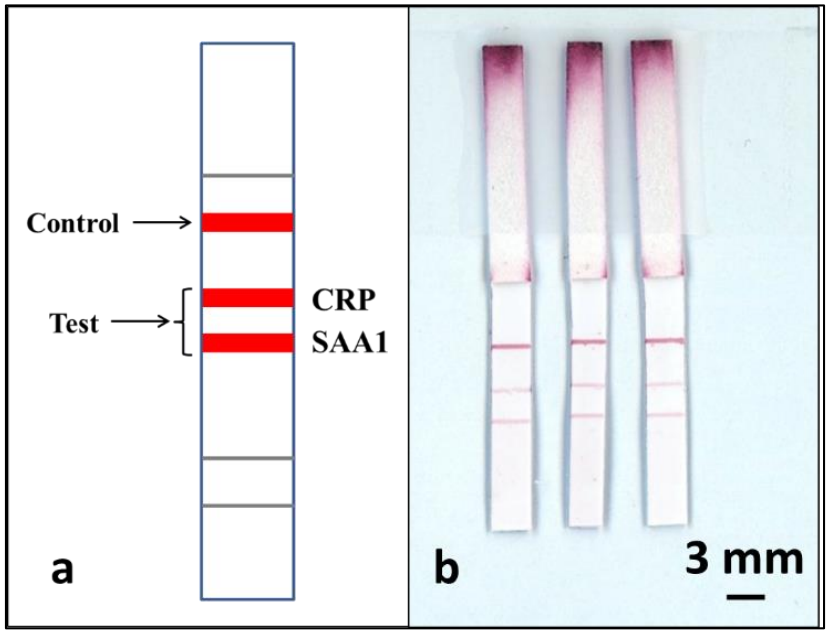
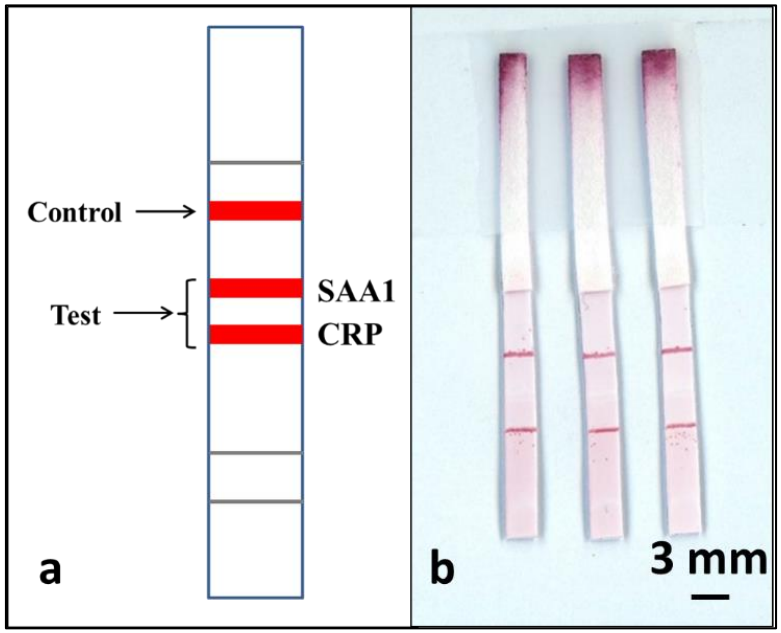
Current method – multiplexing in a single flow path

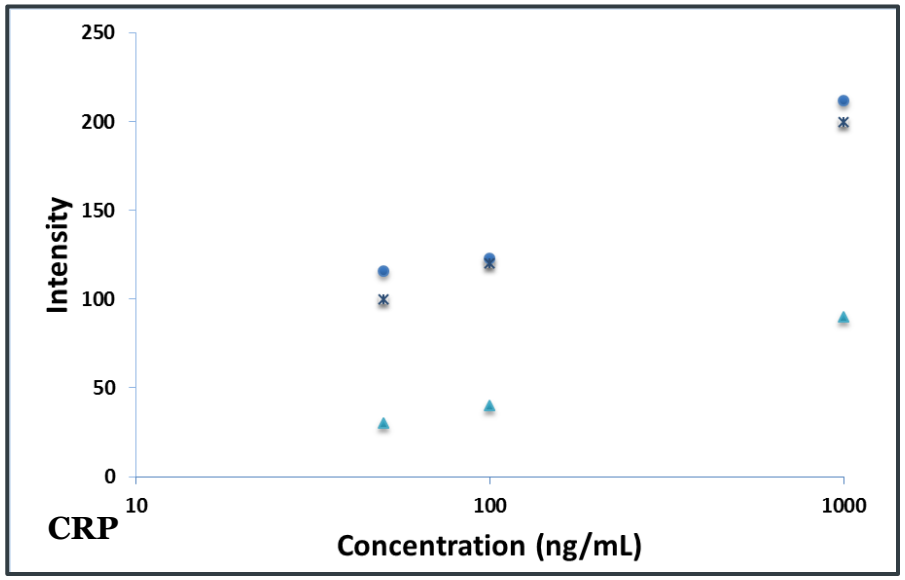


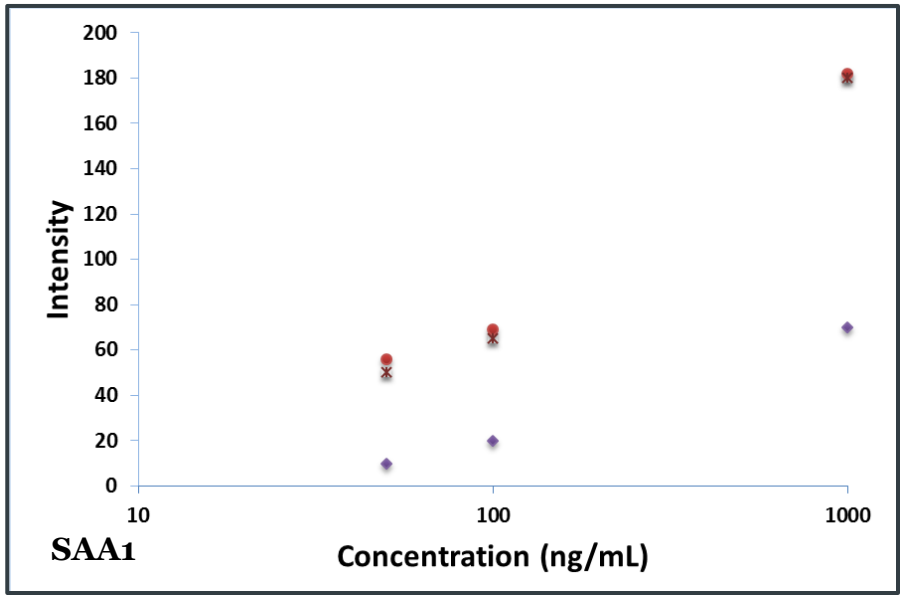


Results for multiplexed detection of CRP and SAA1 in a single LFD with multiple detection sites in the same flow path.

Drawbacks: undesired interference between different detection, namely, the influence of each test-lines on subsequent lines positioned further along the flow-path.

Multiplexed detection on LFDs





CRP SAA1	Standard single LFDs
SAA1	Single-channel LFDs with multiple test lines (CRP before SAA1)
CRP	Single-channel LFDs with multiple test lines (SAA1 before CRP)
CRP SAA1	LDW patterned dual-channel LFDs

The results show that the colour intensity of the test lines (for both CRP and SAA1) for our LDW patterned dual-channel LFDs are at the same level as that for standard single LFDs

Multiplexed detection of Leishmaniasis

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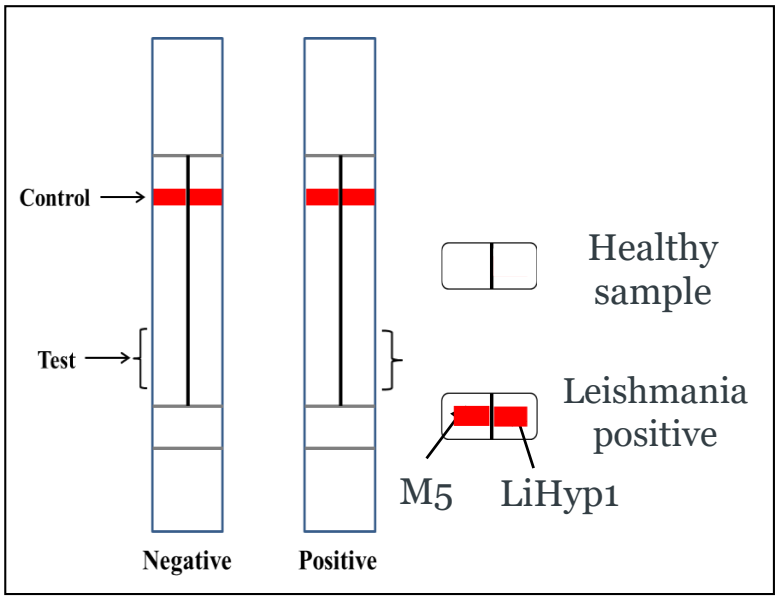
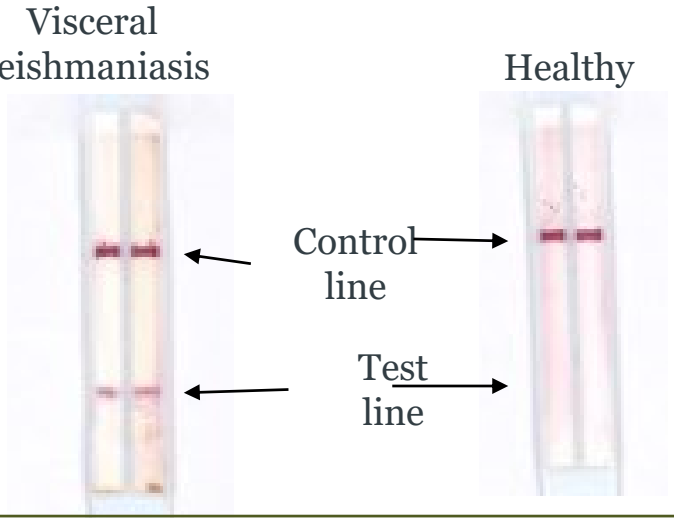


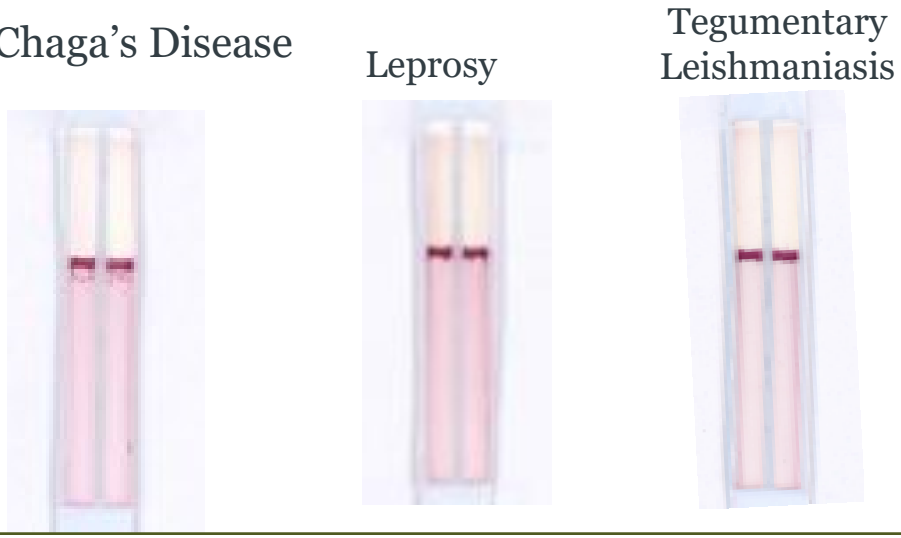
Testing for positive and negative samples:



Samples used – Serum and Whole blood

Healthy negative controls, Visceral Leishmaniasis, Chaga's disease, Leprosis, Tegumentary Leishmaniasis

Testing for cross-reaction:



Improved sensitivity and limit-of-detection on LFDs

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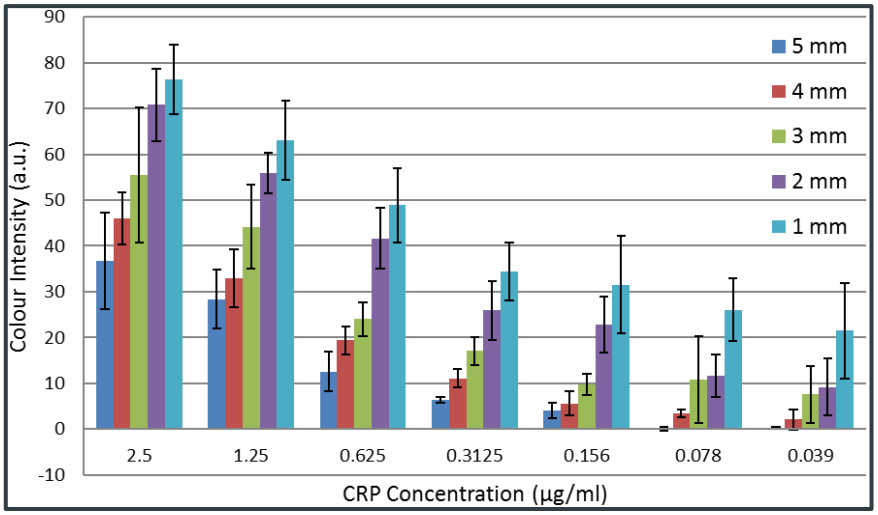
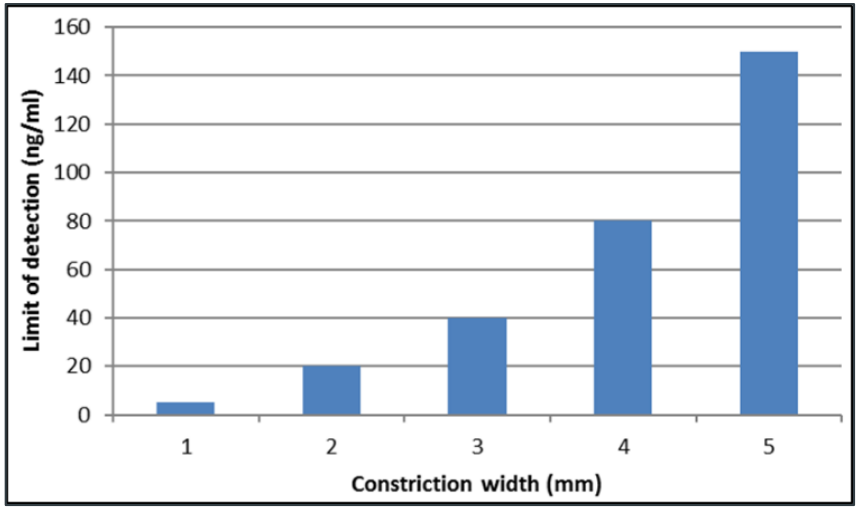
Example– sensitivity and limit-of-detection increase for a CRP assay





A clear signal enhancement can be observed with the decrease of the constriction widths for each concentration.

- Improvement in the sensitivity of the assay by 62x;
- Improvement in the limit of detection by 32x.