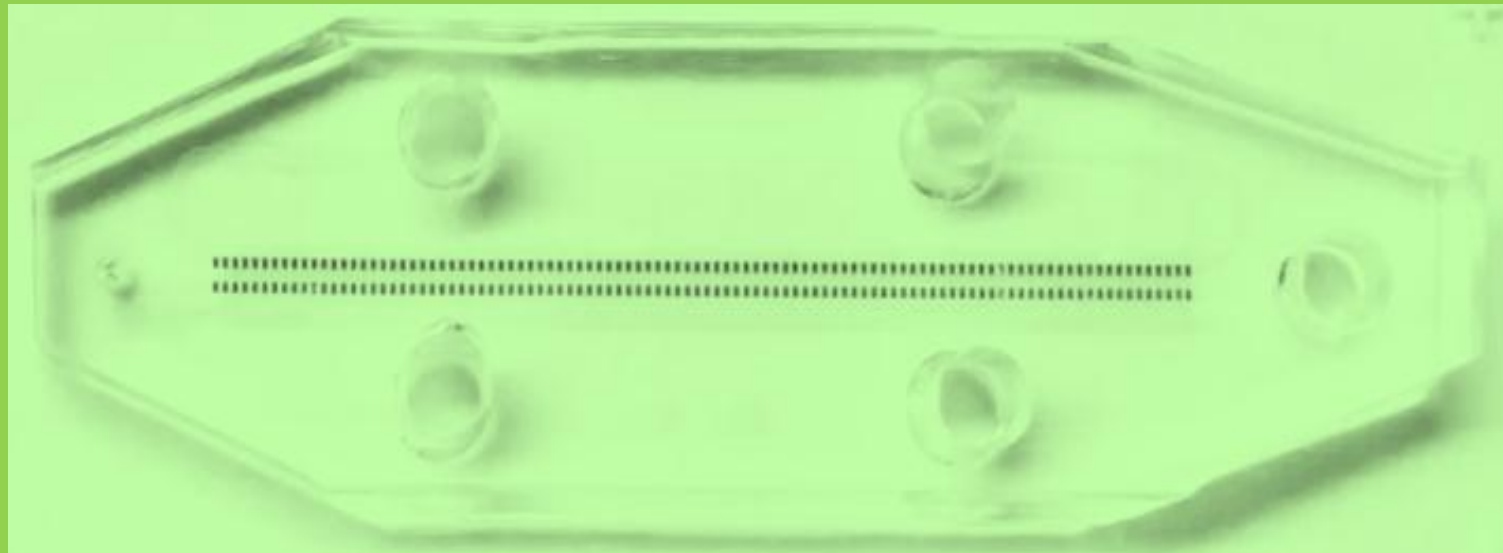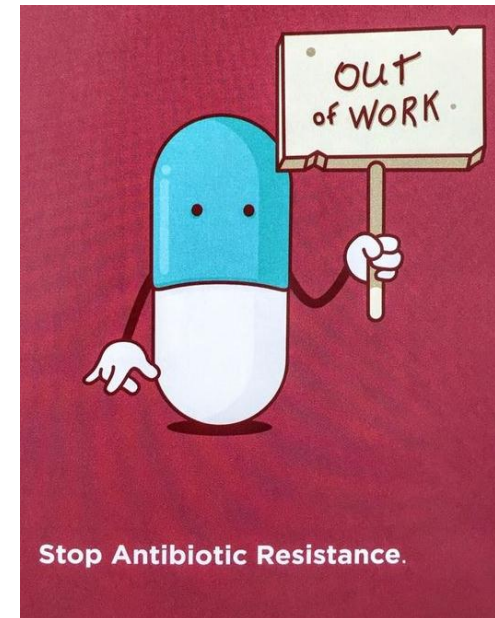بسم الله الرحمن الرحيم

Rapid phenotypic antimicrobial susceptibility testing using nanoliter arrays

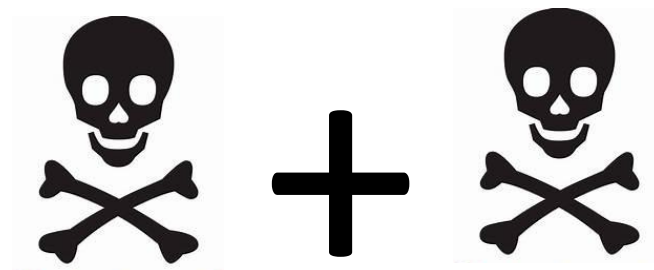# Antibiotic/Antimicrobia resistance (AMR)

Introduction:

infections with **AMR** were estimated to take the lives of over **700,000** people every year, and that number is expected to rise to **10 million** people by the year **2050**

one that is estimated to cost the United States **$35 billion** a year and is expected to cost the world **$100 trillion** by the year 2050.

For Every Hour

Septic Shock

Oxygen Mask

ePainAssist.com

7.6%

This approach facilitates the emergence of AMR in the clinic as well as damage the human microbiota
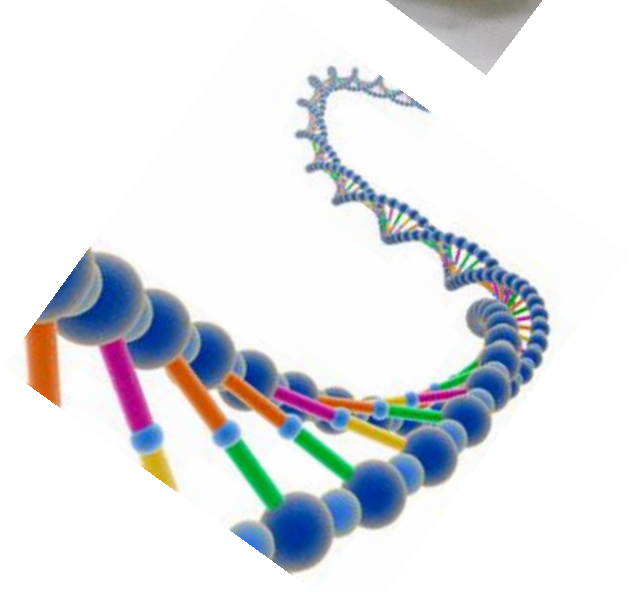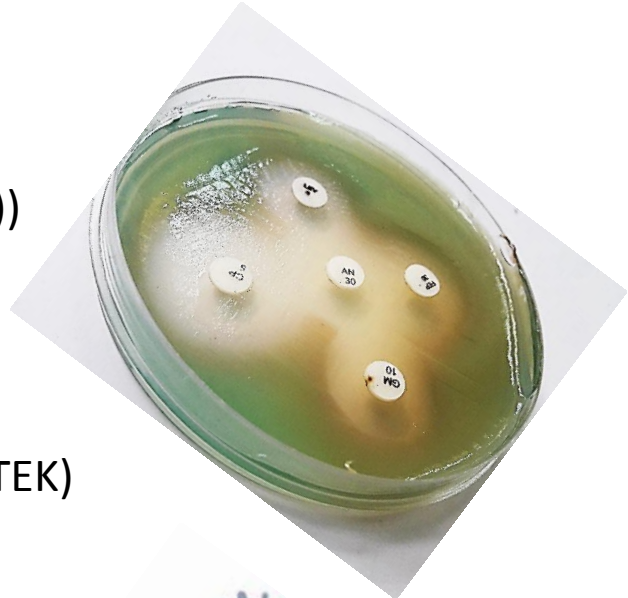
5

Antimicrobial Susceptibility Test methods

- Antibiotics diffusing from paper disks (Kirby–Bauer (disk diffusion))

- Diffusing antibiotic gradients from strips (e.g., E-test)

- Liquid suspension-based methods (broth microdilution method)(VITEK)

- Genotypic AST(Antimicrobial Susceptibility Testing) (1-3h)

Stationary Nanoliter Droplet Array

Herein, we present a system that combines the resazurin assay with a nanoliter well array containing lyophilized antibiotics within each well, forming a system with multiplexing potential that can be operated in a practical manner. Because well volumes are small and chemically isolated, the fluorescence buildup (i.e., metabolism) of a small number of bacterial cells can be detected for different antibiotic conditions simultaneously.
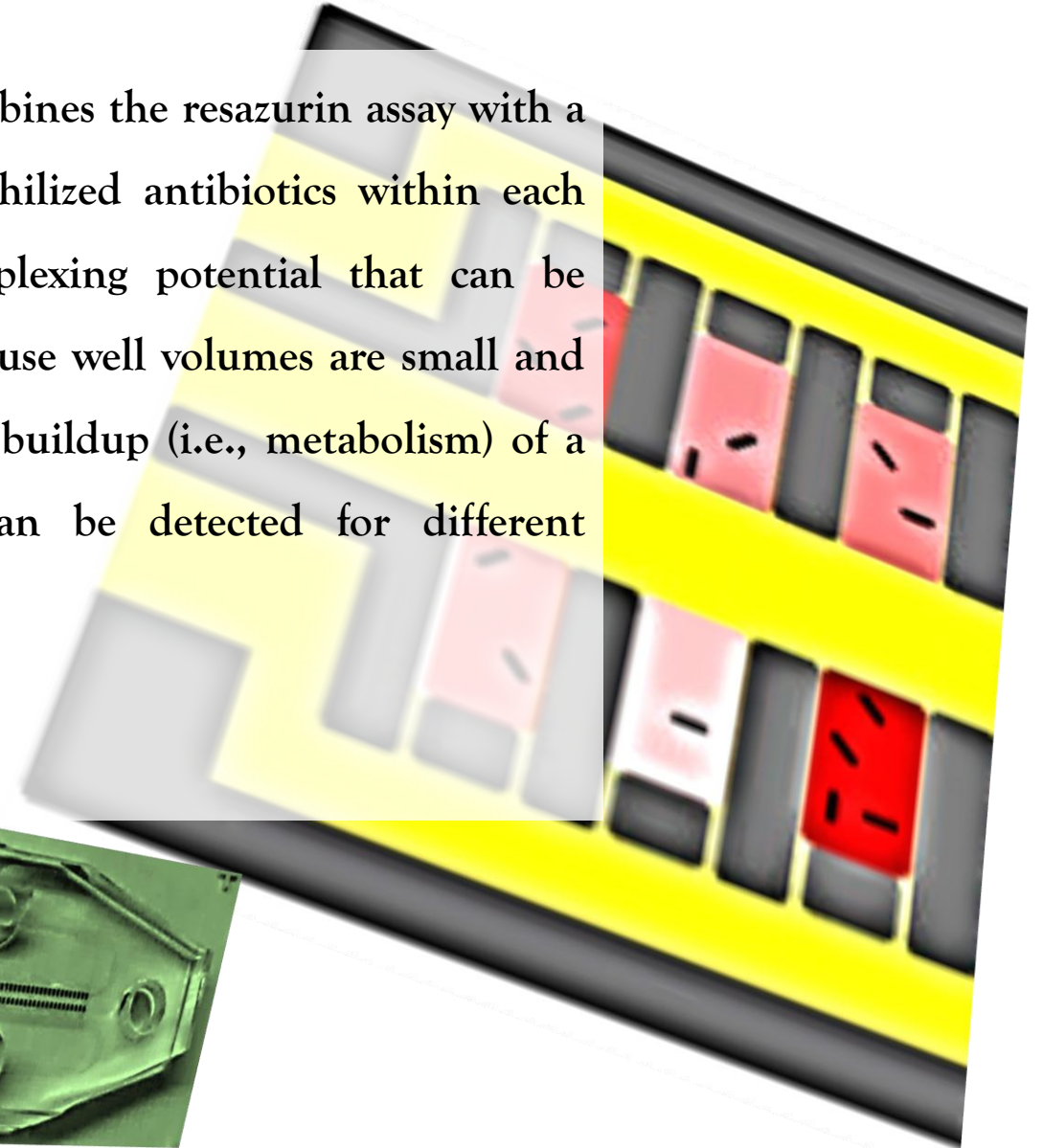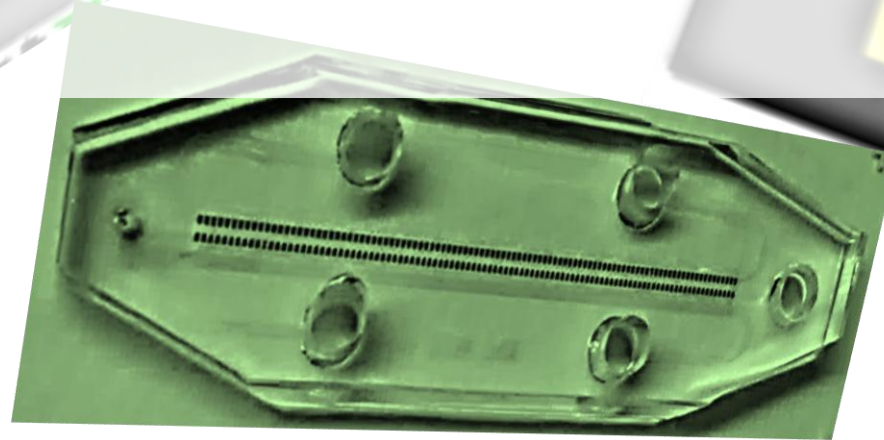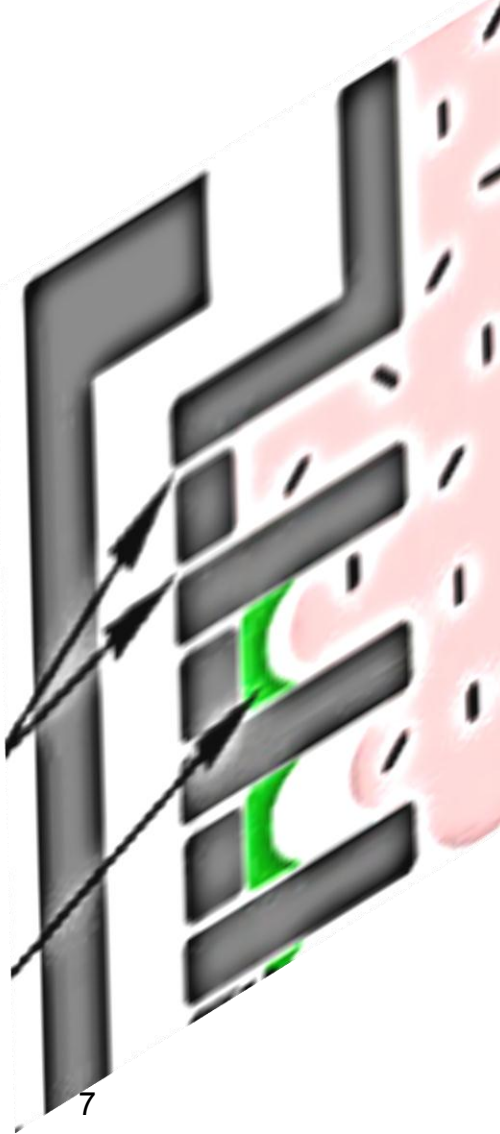
Materials and Methods:

- **Resazurin**
- **FC-40 oil**
- **pipette**
- **bacterial suspension**
- **positive control**
- **negative control**
- **SNDA-AST Device(stationary nanoliter droplet array)**
- **Antibiotic**
- **inverted epifluorescent microscope**

Materials and Methods:

Resazurin ➔ resorufin | reagents

FC-40 oil ➔

Evaporation
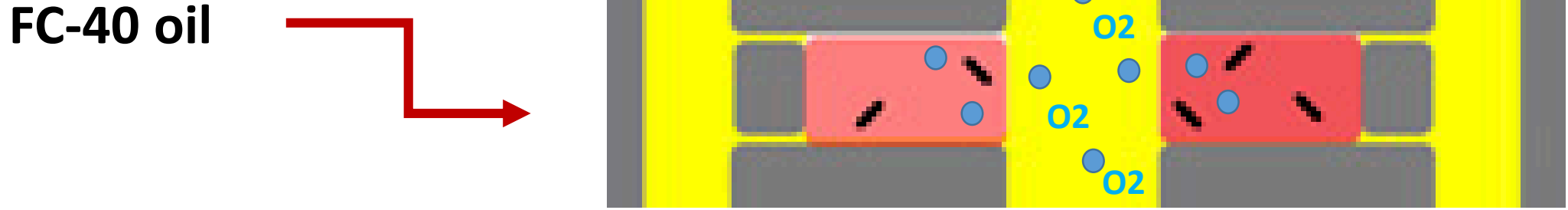
t= 0 min | t= 4 min | t= 8 min
t= 12 min | t= 16 min | t= 20 min
t= 24 min | t= 28 min | t= 32 min
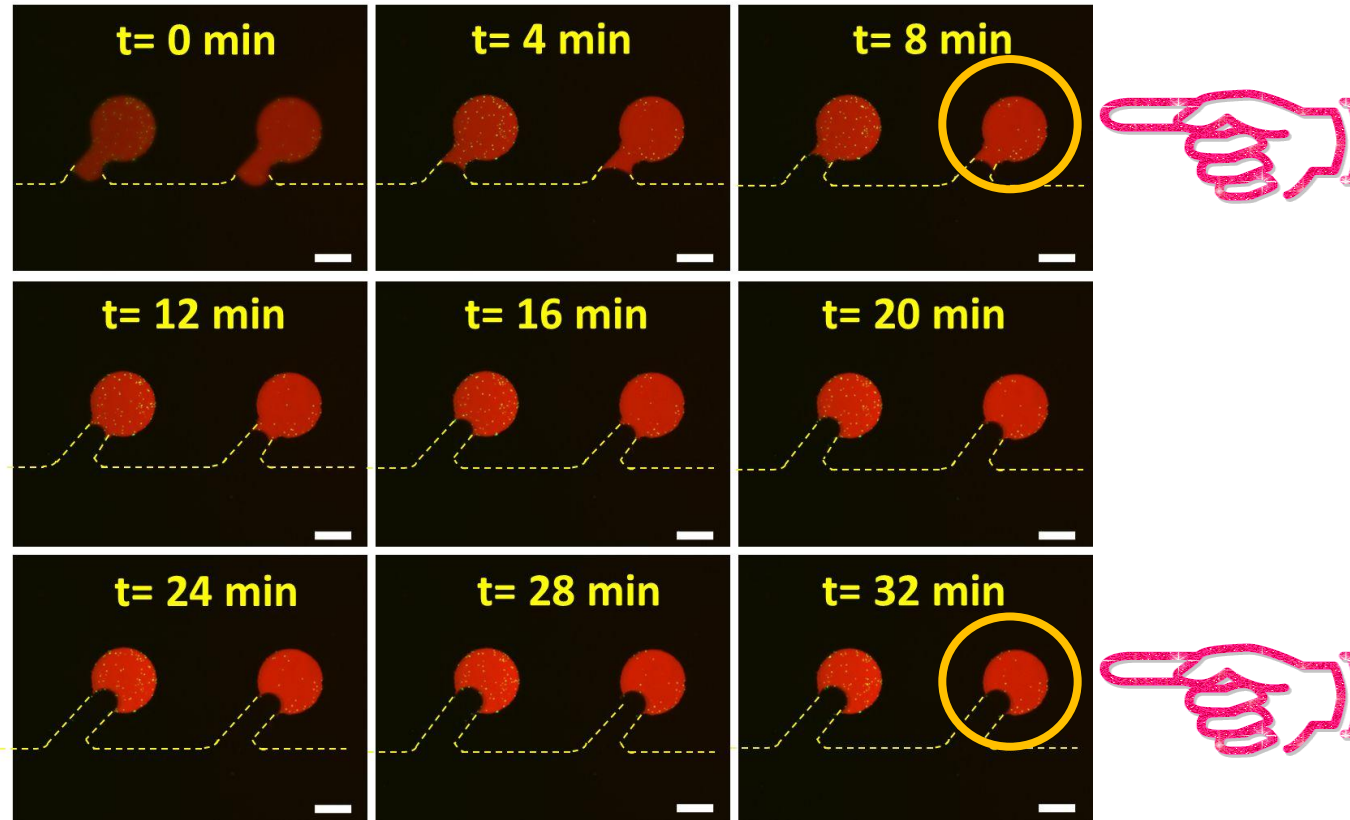
1 nL culture medium (the volume of each microchamber) can be evaporated in less than 1 h(Azizi et.al)

Materials and Methods:

FC-40 oil →

bacterial suspension →

Pipette ⌐

bacterial suspension ⌐

→ 10μL

→ 5×10^5 Cfu/ml

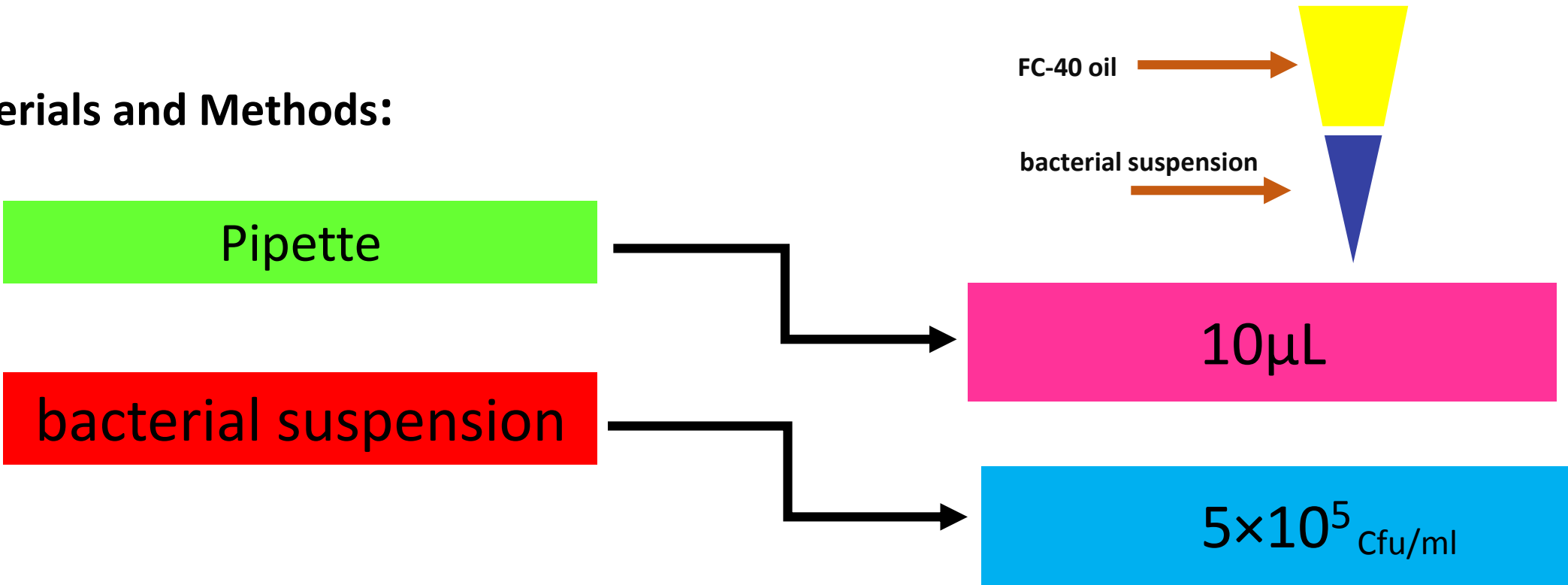positive control → bacterial suspension + Resazurin

Negative control → bacterial suspension + Resazurin + Antibiotics
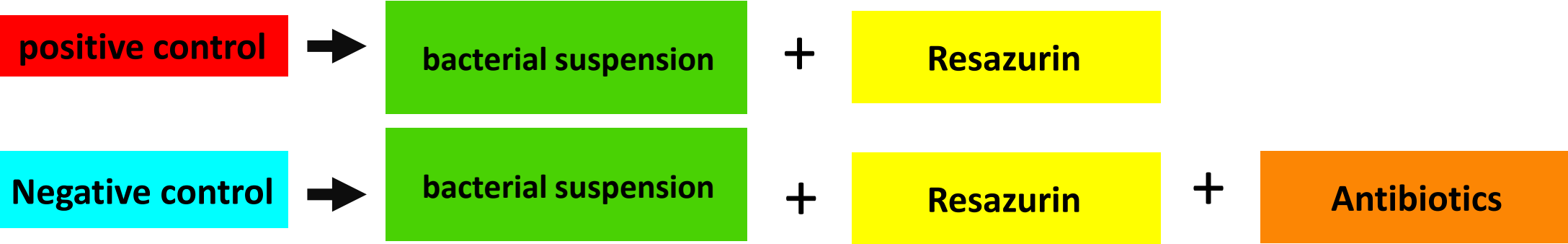
Materials and Methods:

SNDA-AST Device(stationary nanoliter droplet array)

10 μL pipette
oil plug
bacteria with resazurin

Step 1 — Inject bacteria suspension with resazurin — Oil plug follows and separates the wells

Step 2 — 2. Monitor well fluorescence

restrictions
antibiotic

☐ Air ■ Antibiotic ☐ Growth medium with resazurin ■ Reduced resazurin ☐ FC-40 oil

Antibiotic (Lyophilizated)

Load antibiotics into SNDA and freeze (-80°C) for 40 min

Freeze dry (lyophilize) overnight

Prepare Bacteria for seeding at concentrati on of $5*10^5$ CFU/ml

Load bacteria into SNDA (antibiotics redisolve)

B

(B) Comparison of the standard "wet" and dried gentamicin at the breakpoint concentration using E. coli with 8 mg/L ampicillin showing that the dried antibiotic has efficacy similar to that of the standard "wet" counterpart.
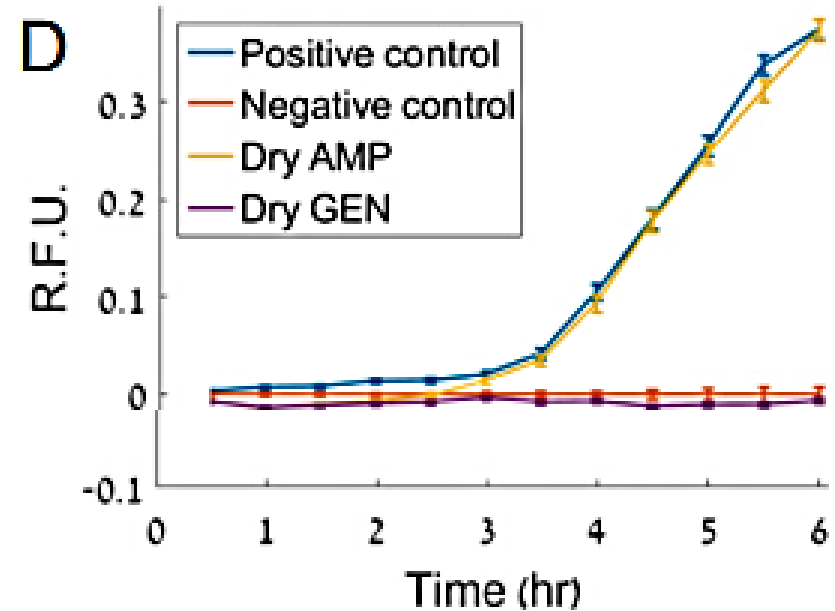
D

(C) Schematic of parallel SNDA–AST device used for multiplexing. A bacterial sample can be loaded into the device and tested against two different types of antibiotics simultaneously.
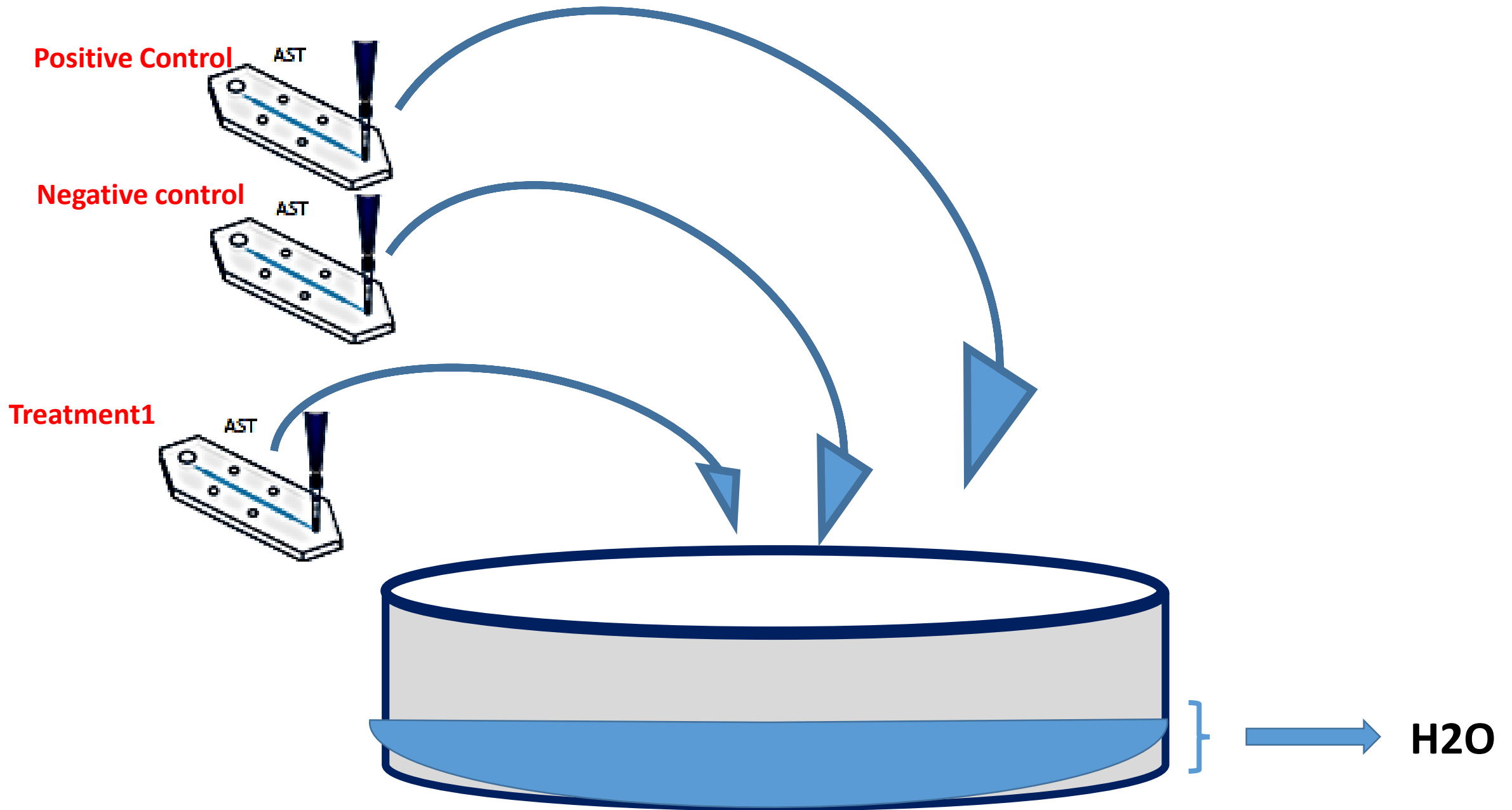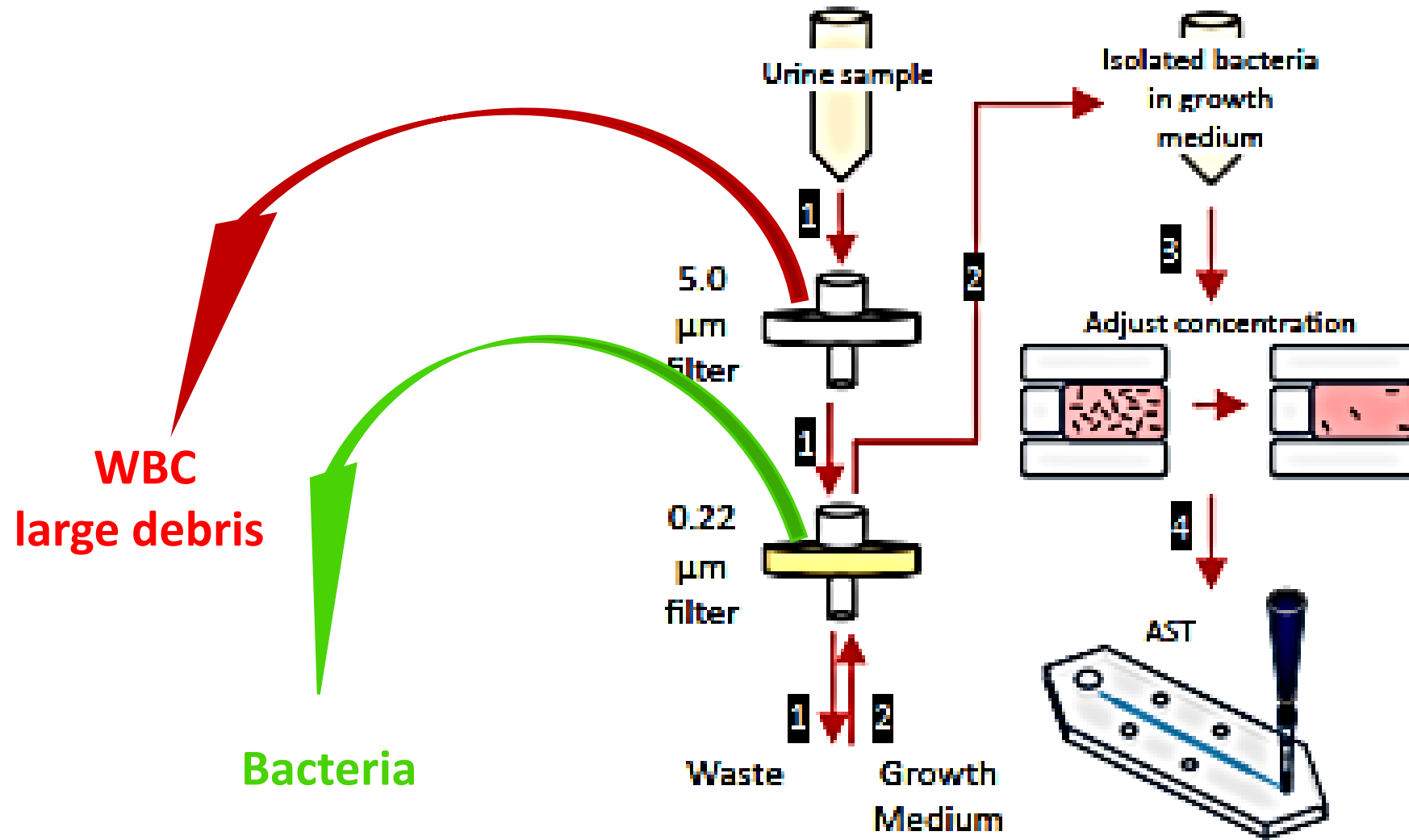
Positive Control

AST

Negative control

AST

Treatment1

AST

H2O

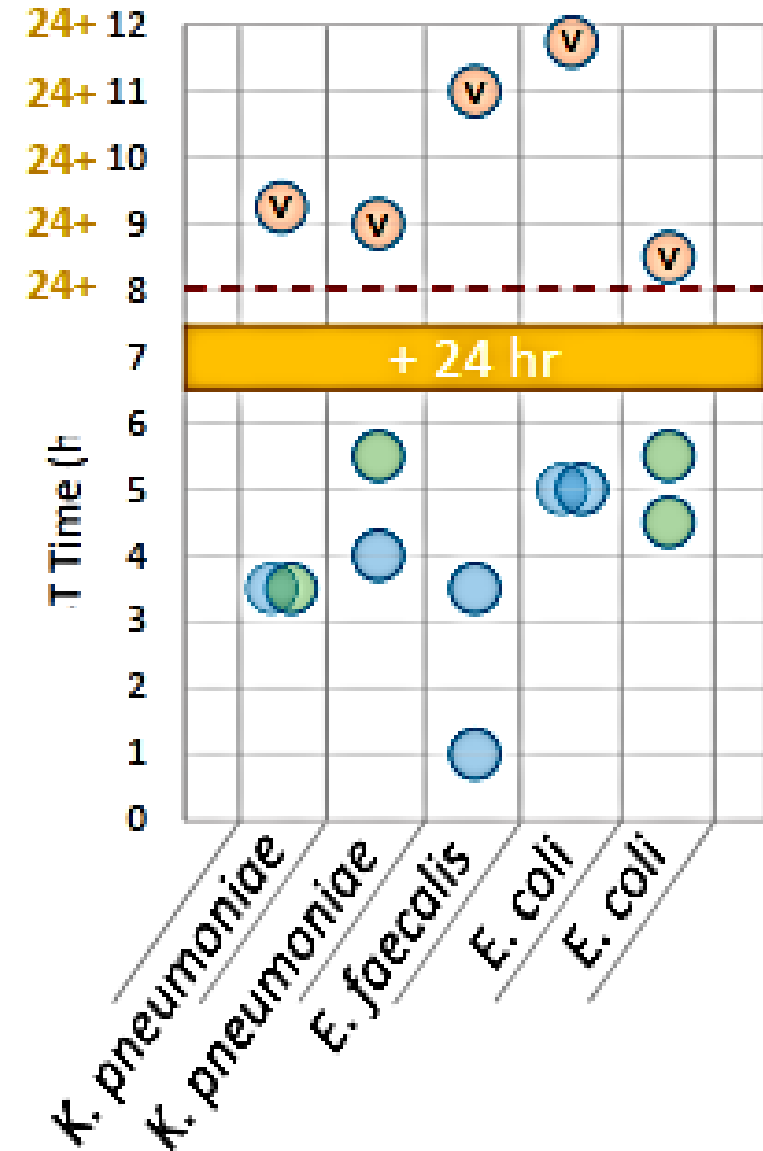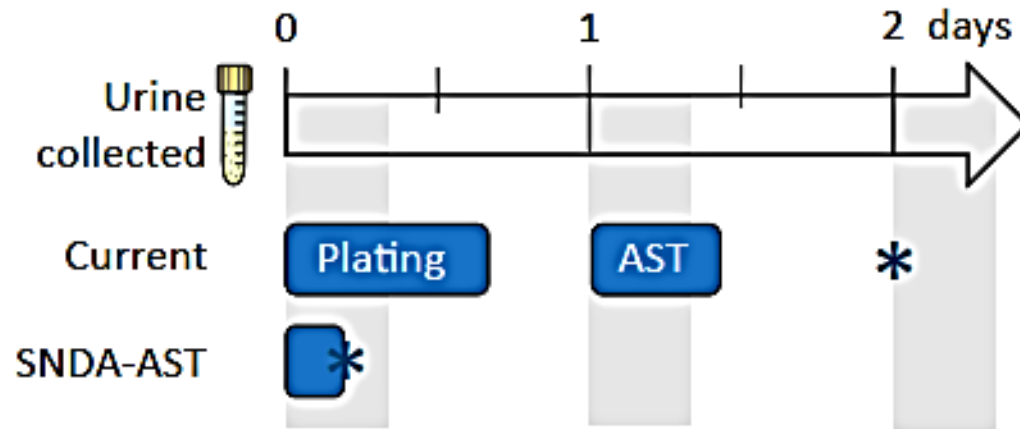**Table 1.** Experiments used to estimate the time to S/R determination of the SNDA–AST device

Bacteria	Isolate	Source	SNDA–AST determination (time)	VITEK 2 (laboratory) determination (time)
E. coli	Clinical	Urine	AMP 8 mg/L – S (5.00 h) CIP 0.5 mg/L – R (4.00 h)	AMP – S CIP – R (9.75 h)
K. pneumoniae	Clinical	Urine	AMP 8 mg/L – R (4.50 h) GEN 2 mg/L – S (2.00 h)	AMP – R GEN – S (8.75 h)
Staphylococcus aureus	Clinical	BAL	PEN 0.125 mg/L – R (2.50 h) CIP 1 mg/L – S (4.00 h)	PEN – R CIP – S (9.75 h)
Staphylococcus haemolyticus	Clinical	Urine	CIP 1 mg/L – S(5.35 h) ERY 1 mg/L – S (3.85 h)	CIP – S ERY – S (9.75 h)
Acinetobacter baumannii	Clinical	Burn	CIP 1 mg/L – S (4.25 h) CST 2 mg/L – S (2.25 h)	CIP – SCST – S (9.25 h)
Citrobacter freundii	Clinical	Urine	CIP 1 mg/L – S (4.50 h) GEN 1 mg/L – S (4.50 h)	CIP – S GEN – S (9.50 h)

Same-Day Detection and AST for UTIs

WBC
large debris

Bacteria

Urine sample

Isolated bacteria
in growth
medium

5.0 µm filter

0.22 µm filter

Adjust concentration

Waste   Growth Medium

AST

# Same-Day Detection and AST for UTIs

Experiments used to estimate the time to S/R determination of the SNDA–AST device

Exp.	Organism	SNDA–AST determination (time)	VITEK 2 (laboratory) determination (time)
1	*K. pneumoniae*	AMP 8 mg/L – R (3.5 h) CIP 0.5 mg/L – S (3.5 h)	AMP – R CIP – S (9.25 h)
2	*K. pneumoniae*	AMP 8 mg/L – R (5.5 h) CIP 1 mg/L – S (.0 h)	AMP – R CIP – R (.0 h)
3	*Enterococcus faecalis*	AMP 8 mg/L – S (1.0 h) CIP 4 mg/L – S (3.5 h)	S – AMP S – CIP (11.0 h)
4	*E.coli*	AMP 8 mg/L – S (.0 h) CIP 0.5 mg/L – S (5.0 h)	R – AMP S – CIP (11.75 h)
5	*E. coli*	AMP 8 mg/L – R (4.5 h) CIP 0.5 mg/L – R (5.5 h)	R – AMP R – CIP (8.5 h)

Schematic of parallel SNDA–AST device used for multiplexing
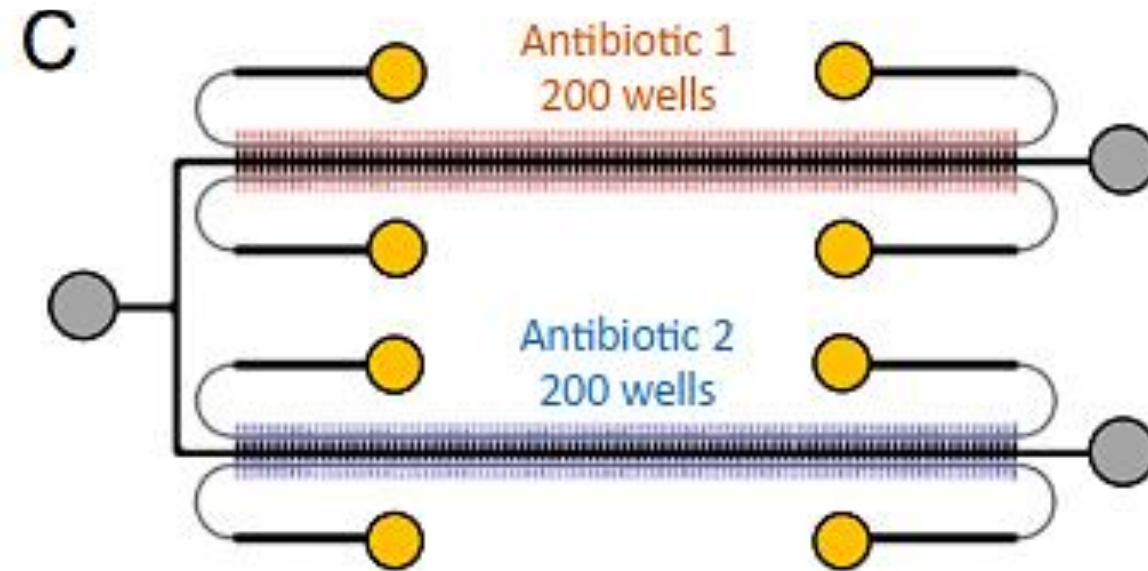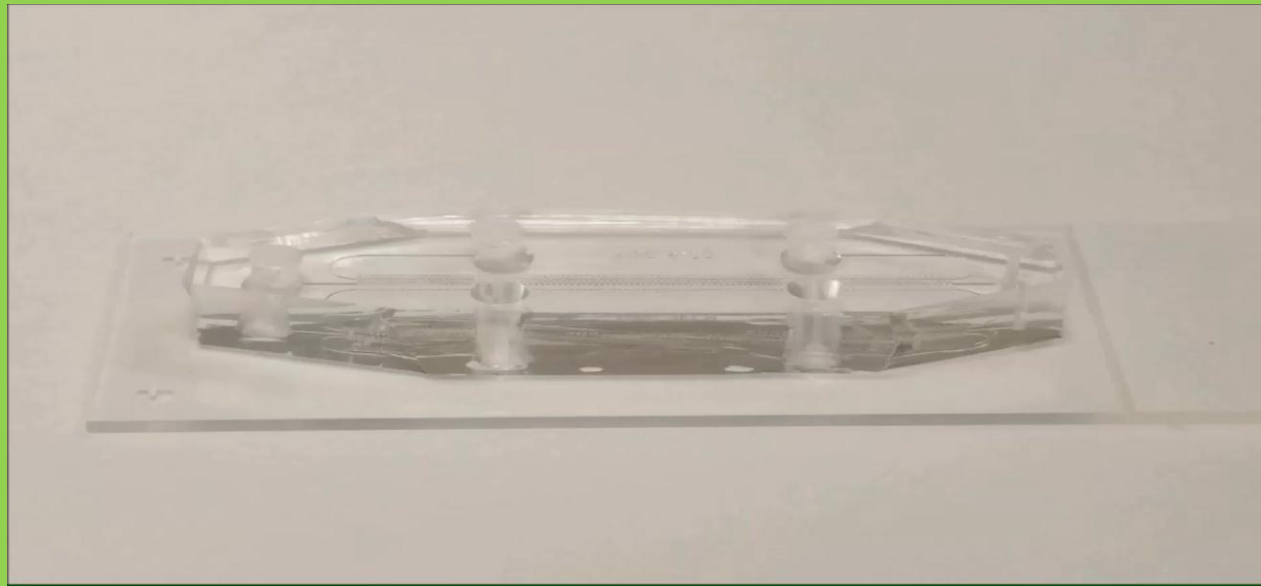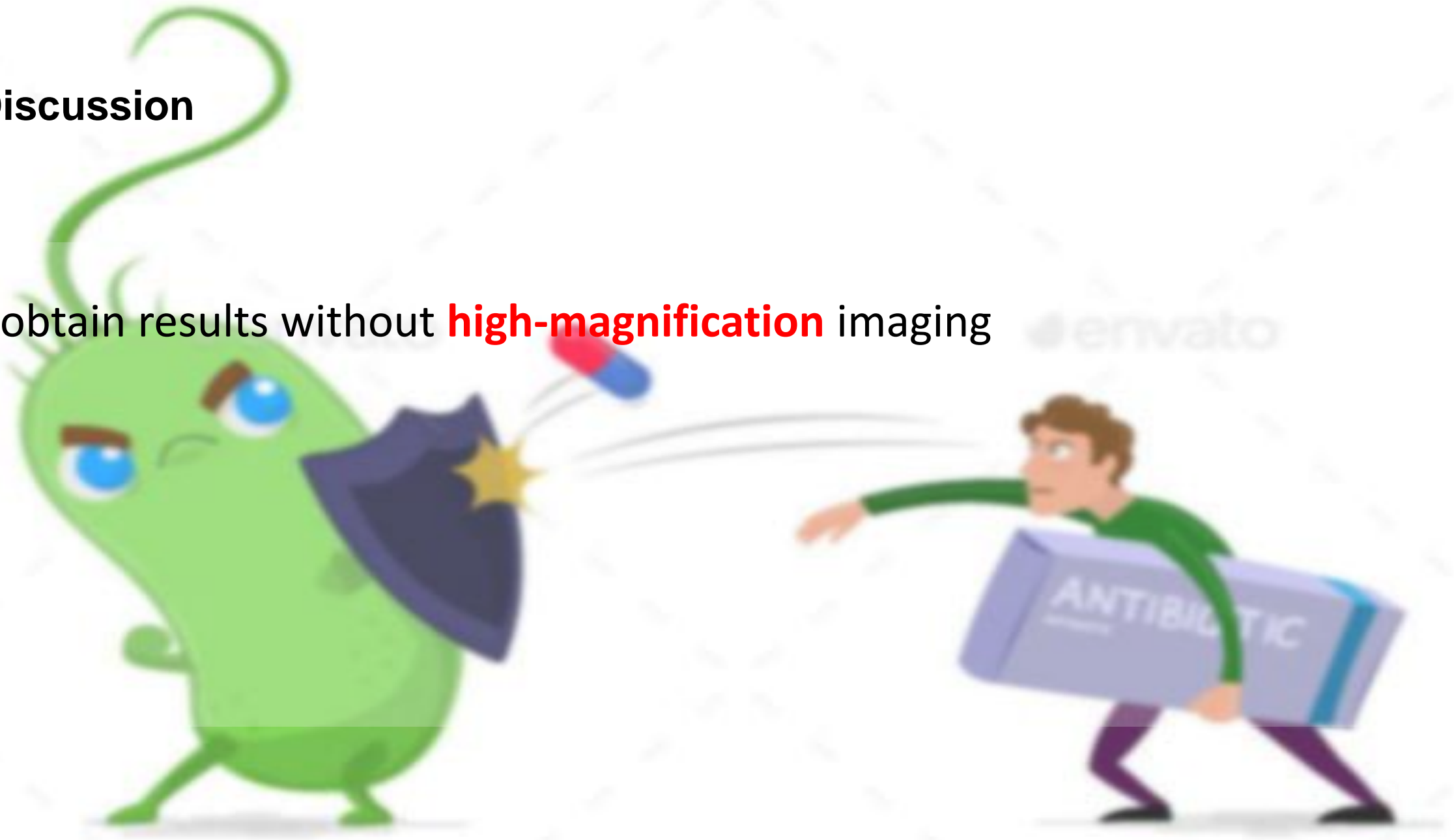
Sensitivity (%)	Specificity (%)
100	99.3

Specificity
(%)

9.3

Discussion

➢ **Same-day** AST results are possible using the SNDA–AST system

➢ The use of **resazurin** as a reporter**/**optical density-based systems

➢ the low reagent consumption of the system**, <1 µL** per test treatment

➢ Urine **filtration** method **/**plating

Discussion

➢obtain results without **high-magnification** imaging

# Rapid phenotypic antimicrobial susceptibility testing using nanoliter arrays

Jonathan Avesar[a], Dekel Rosenfeld[a], Marianna Truman-Rosentsvit[b], Tom Ben-Arye[a,c], Yuval Geffen[d], Moran Bercovici[b], and Shulamit Levenberg[a,c,1]

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Nanoliter-Sized Microchamber/Microarray Microfluidic Platform for Antibiotic Susceptibility Testing

Morteza Azizi, Meisam Zaferani, Belgin Dogan, Shiying Zhang, Kenneth W. Simpson and Alireza Abbaspourrad*

analy
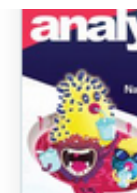
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طالِبُ العِلمِ مَحفوفٌ بِعنایَةِ اللَّهِ؛
عنایت خداوند جویای دانش را فرا گرفته است.

حکمت نامه پیامبر اعظم(ص)، ج ۱، ص ۳۱۳، ح ۳۰۹

Thanks For Your Attention