Next generation methods of AB susceptibility testing

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For a new test, what are your

Expectations?
Needs?
Wishes?
Outcomes?

https://youtu.be/plVk4NVIUh8

AB susceptibility testing: new techniques or next generation AST

Expectations, needs, outcomes:

- High performance (EA, CA, reproducibility, repeatability,..)
- High sensitivity (low bacterial loads)
- Directly from sample
- Minimal inhibitory concentration (MIC)
- Large panel of Antibiotics
- Detection of resistance mechanisms
- Rapid
- High throughput
- Automated (minimal hands-on time)
- Miniaturised (nanotechnology)
- Low cost (high cost → patient selection)
- Laboratory impact?
- Clinical impact?
- Added value?

AB susceptibility testing: new techniques or next generation AST

Newly available technics

- **rapid nucleic acid amplification tests (NAAT) integrating panels for resistance genes**
  - *BDmax, GeneXpert, Amplex-Easyplex*
- **rapid immunochromatography assay panels for detection of resistance genes**
  - *NG Biotech (NG-Test Carba5, NG-Test CTX-M, NG-Test MCR-1)*
- **next-generation sequencing (NGS) → analytical pipelines for resistance markers (resistome)**
- **MALDI-TOF**
- **real-time microscopic growth observation (single cells measurements)**
  - *Accelerate Diagnostics, dRAST -Quantamatrix*
- **early growth disk diffusion reading (EUCAST)**
- **lab automation for manual and/or automated AB susceptibility testing**
  - *BD Kiestra (IdentifA/SusceptA), WaspLab (Colibri)*
- **flow cytometry (FC)**
- **light scattering**
  - Alifax

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- early growth disk diffusion reading (EUCAST)
- **lab automation for manual and/or automated AB susceptibility testing**
- **flow cytometry**
- light scattering

real-time microscopic growth observation (single cells measurements)

https://ssidiagnostica.com/nordic/solutions/automation/drast/

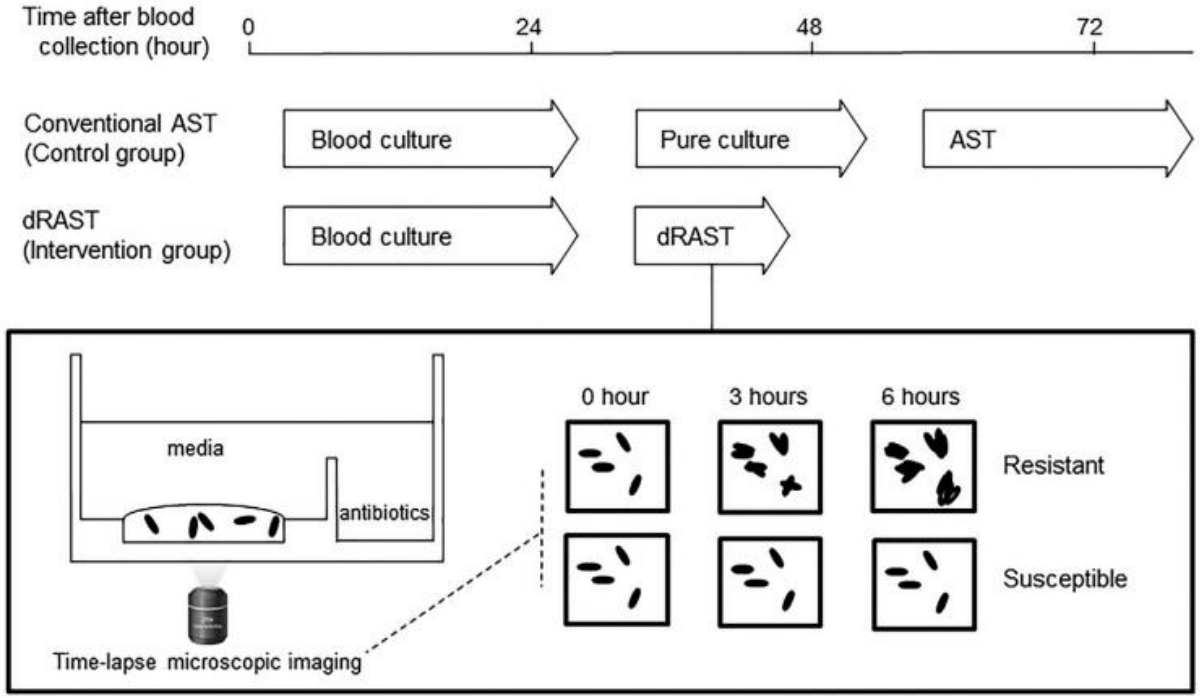


Fig. 1. Process of blood culture in the control and intervention groups. Timeline was adjusted based on actual practice in this study. AST, antimicrobial susceptibility test; dRAST, direct rapid antimicrobial susceptibility testing.

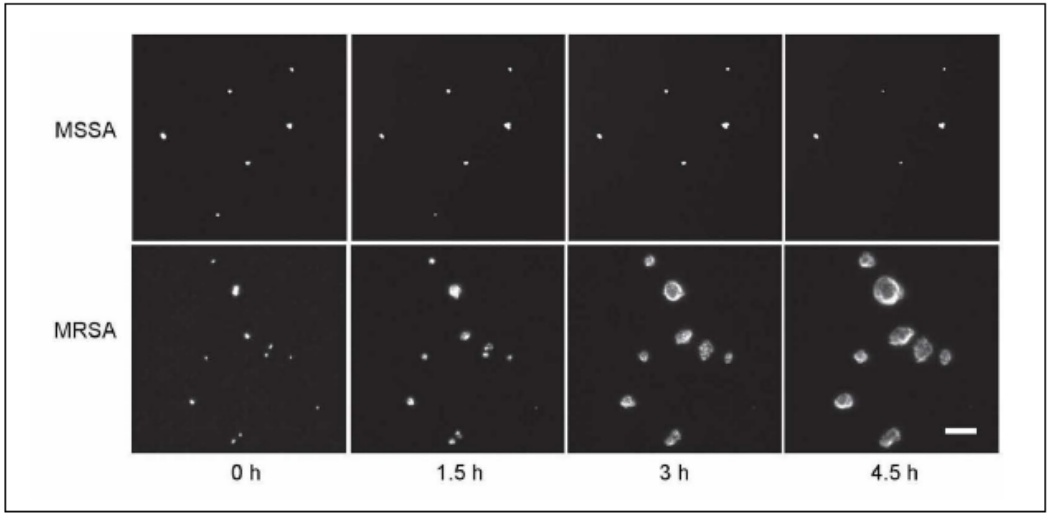
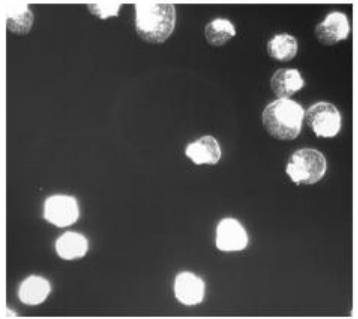
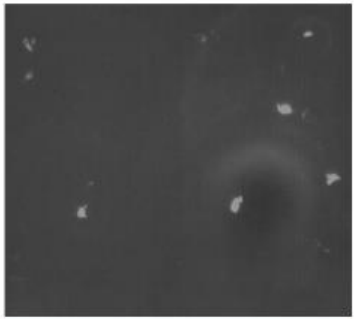


Figure 5. Time-lapse images of methicillin-susceptible *S. aureus* (MSSA) and MRSA isolates growing in cefoxitin at 0, 1.5, 3, and 4.5 hours. By 4.5 hours, susceptible clones have arrested or lysed, while resistant clones continue to grow. The images are enlarged to view individual bacterial clones. Scale bar in lower right image, 20 µm.



E. coli challenged by Ceftazidime with a demonstrated MIC of 32

Resistant (R)



E. coli challenged by Ceftazidime with a demonstrated MIC of 2

Susceptible (S)

http://acceleratediagnostics.com/products/the-accelerate-ruo-clinical/

LAB automation

Image (Copan)

Image (BD Kiestra)

Automated colony picking

↓

Bacterial suspension

↓

ID: MALDI plate

AST
Cards
Disk diffusion

Automated ID/AST

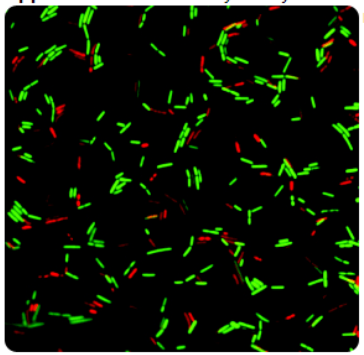
- Improved quality, accuracy, reproducibility, traceability
- Reduced hands-on time
- Additional components to real “full automation”

Image analysis software for quantification of bacterial growth

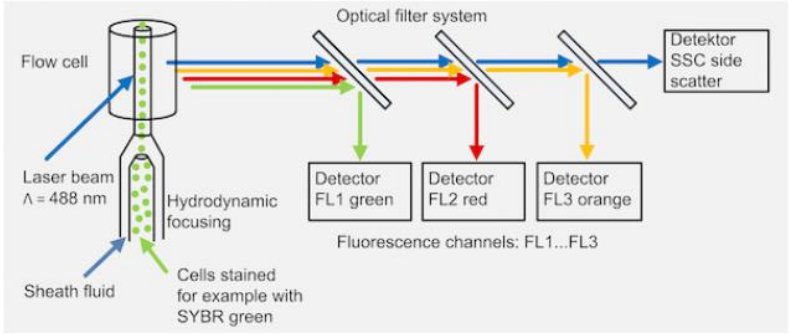
- Automated reading of disk diffusion (Etest?)

Flow cytometry

Applications Cell viability assay

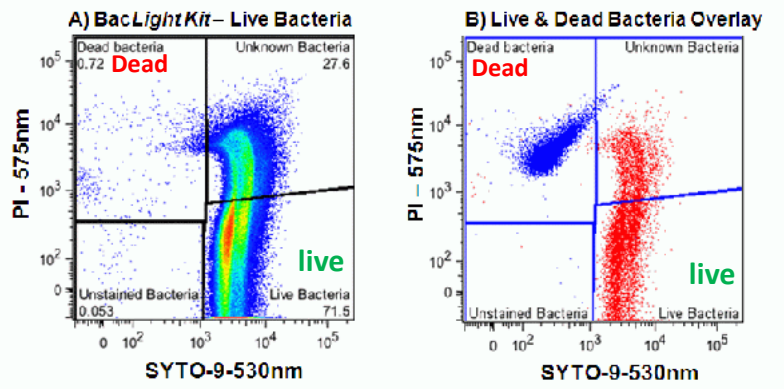


http://www.genecopoeia.com/product/viaquant-viabilitycytotoxicity-kit-for-bacteria-cells/



http://www.metanor.com/en/product/the-online-bacteria-analyzer.html

Biparametric histogram



https://www.qmul.ac.uk/blizard/research/lab-management-and-core-facilities/flow-cytometry/uses-of-flow-cytometry/bacteria/

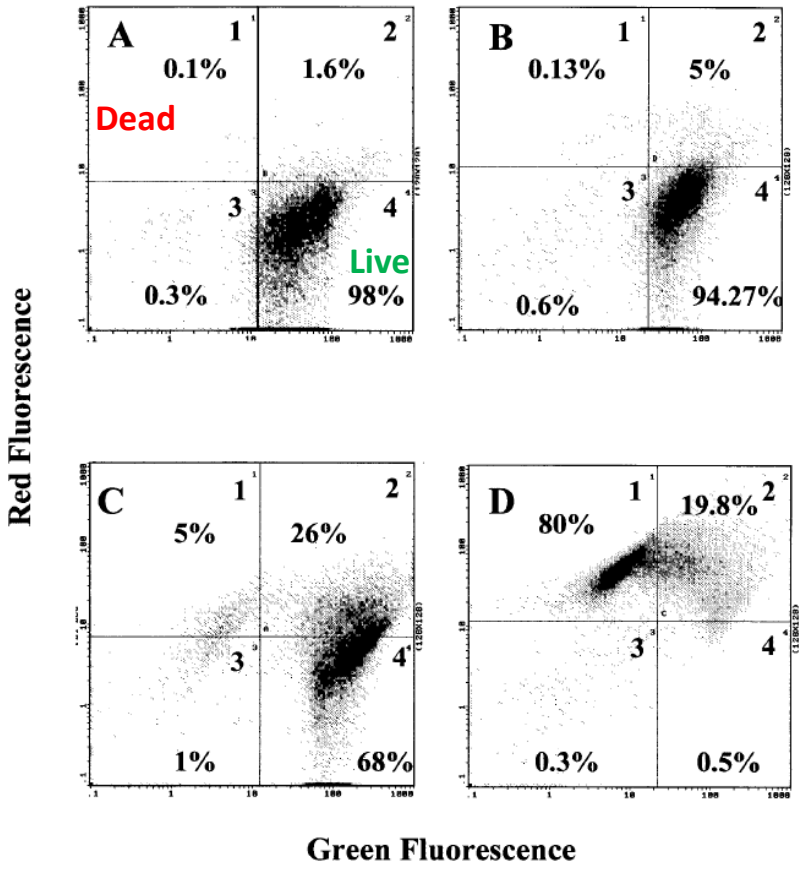
SYTO: green fluorescent nucleic acid stain

- Live and dead bacteria

Propidium iodide: red fluorescent nucleic acid stain

- Dead bacteria

Antimicrobial susceptibility testing by FCM using the Bac/live kit (Molecular Probes)



A: *E. coli* without AB
B: *E. coli* + vancomycin (2h / 1.024 g/ml)
C: *E. faecium* VRE + vancomycin (2h / 1.024 g/ml)
D: *E. faecalis* + vancomycin (2h / 1.024 g/ml)

Detection of resistant/tolerant subpopulations

Alvarez-Barrientos et al. *Clinical Microbiology Reviews*. 2000.

FASTinov
Next Generation
Antimicrobial Assays

FAST SUSCEPTIBILITY TESTING
FOR ACUTE CARE
DIRECTLY FROM POSITIVE BLOOD CULTURES

OUR DEVICE

FASTgramneg
(gram negative bacteria)

FASTgrampos
(gram positive bacteria)

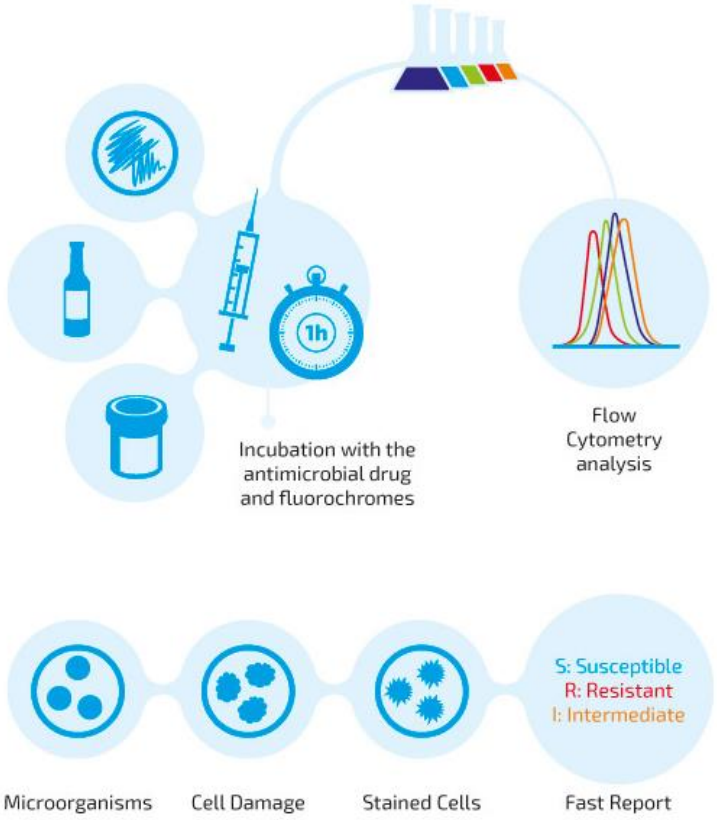
FASTyeasts

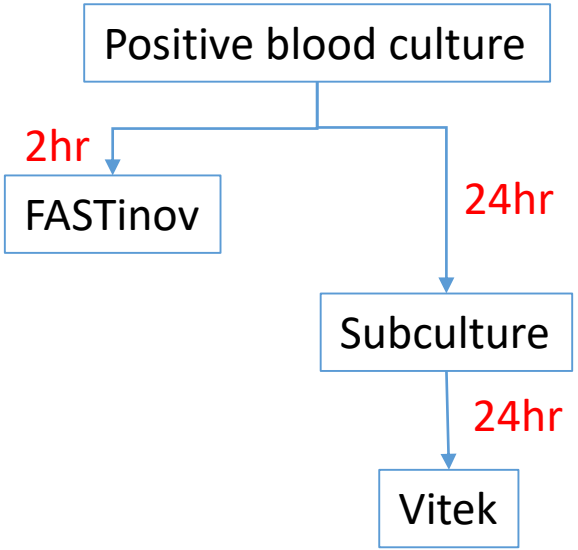
FASTmar
(mechanisms of antimicrobial resistance)

+

Dedicated decision-support software according to CLSI and EUCAST protocols

- Microplate with lyophilized antimicrobials
- Specific fluorochrome
- Dedicated software
- FC (not provided!!)





Potential impact of FC AST on the clinical management of GN bacteremia using FASTinov

102 patients

- 16.7% of patients received initially inappropriate therapy.
  - ➢ mean hospital length was significantly higher

TABLE 3 | Categorical agreement between FASTinov® Gram negative test or Vitek2 (bioMérieux) and broth microdilution; FASTinov ® gramneg kit error rate.

Agreement with microdilution	FASTinov®	Vitek2[a]	No. (%) mE	95% CI for mE (%)	No. (%) ME	95% CI for ME (%)	No. (%) VME	95% CI for VME (%)	Broth microdilution AST	
									Susceptible	Resistant
Amikacin	0.97	0.93	2 (1.55)	(0.19: 5.49)	2 (1.77)	(0.21: 6.25)	–	–	113	16
Amoxicillin+ clavulanic acid	0.97	0.94	–	–	3 (3.80)	(0.79: 10.70)	1 (2.00)	(0.05: 10.65)	79	50
Cefotaxime	0.98	1.00	1 (0.78)	(0.02: 4.24)	1 (1.10)	(0.03: 5.97)	–	–	91	38
Ceftazidime	0.97	0.94	2 (1.55)	(0.19: 5.49)	2 (2.17)	(0.26: 7.63)	–	–	92	37
Ciprofloxacin	0.94	0.96	5 (3.88)	(1.27: 8.81)	3 (3.57)	(0.74: 10.08)	–	–	84	45
Colistin	1.00	1.00	–	–	–	–	–	–	126	3
Gentamicin	0.98	0.96	–	–	1 (1.08)	(0.03: 5.85)	1 (2.78)	(0.07: 14.53)	93	36
Meropenem	0.98	1.00	1 (0.78)	(0.02: 4.24)	1 (0.88)	(0.02: 4.83)	1 (6.25)	(0.16: 30.23)	113	16
Piperacillin+ tazobactam	0.98	0.95	1 (0.78)	(0.02: 4.24)	1 (1.15)	(0.03: 6.24)	–	–	87	42
Overall	0.98	0.97	12 (1.03)	(0.54: 1.80)	14 (1.59)	(0.87: 2.66)	3 (1.06)	(0.22: 3.07)	878	283
ESBL I detection	1.00	1.00								

[a]*Result obtained from colonies.*

mE, minor errors; ME, major errors; VME, very major errors;–, no error; CI, confidence interval.

Costa-de-Oliveira et al. *Frontiers in Microbiology*. 2017

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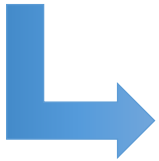
- Rapid AST (1-4h)
- High correlation between microdilution and FC MIC
- Rapid identification of resistance mechanisms (ESBL,….)
- Bacteria (Mycobacteria) and Yeast



- Rapid AST (4-10 days ??) → Conventional (=> 14 days)
- Killing kinetics / rates
- Characterize subpopulations
- Can be performed outside containment facilities --> formaldehyde fixed cells

Hendon-Dunn, C. et al. *Antimicrobial Agents and Chemotherapy*. 2016

-

- Choice of appropriate Phenotypic markers (the best markers are those associated with the irreversible impairment of cell viability)
- Throughput

AB susceptibility testing: new techniques or next generation AST

Potential next generation AST technics

- Atomic force microscopy (cantilever)
- Magnetic bead rotation
- Microfluidics and microdroplets methods
  - nanoliter arrays (microfluidic)
  - microdroplets
- Small molecule sensor array (optoelectronic nose)
- Multiplexed bead-based bioassay
- Isothermal micro-calorimetry
- Optical tweezers
- Biomimetic polymer sensor
- Raman spectroscopy

Schumacher et al. *Eur J Clin Microbiol Infect Dis*. 2018
van Belkum et al. *JCM*. 2013

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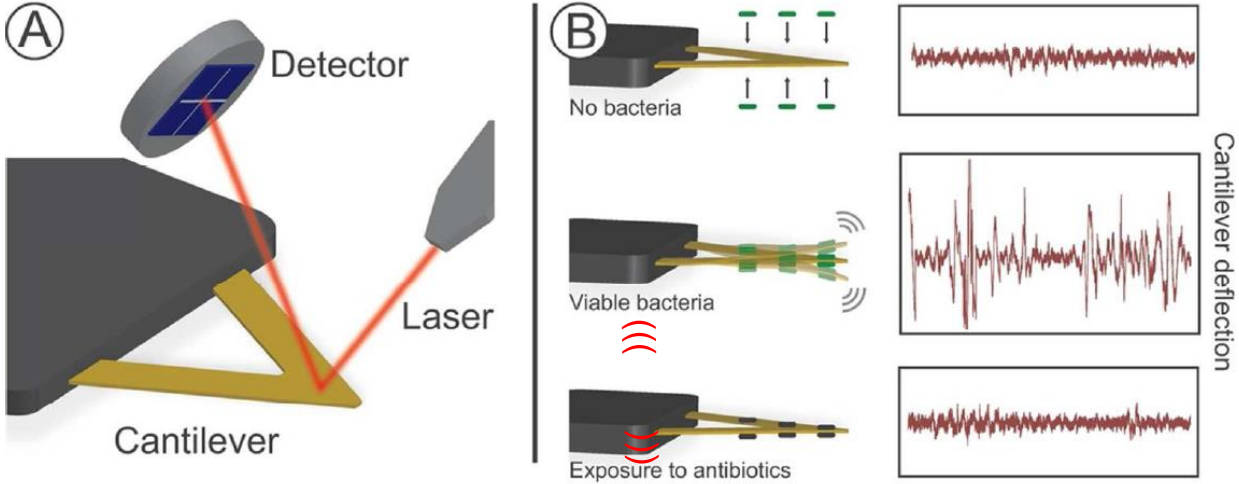
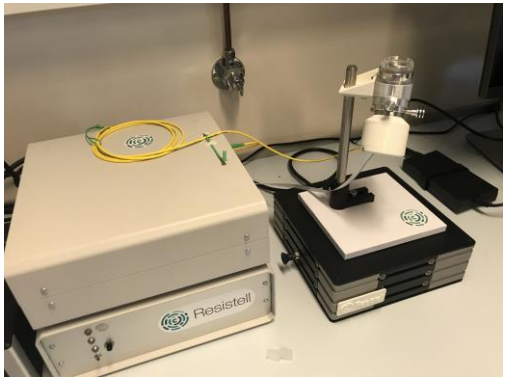
Atomic force microscopy (AFM)

Rapid detection of bacterial resistance to antibiotics





Stupar, P., O. Opota, G. Longo, G. Prod'hom, G. Dietler, G. Greub and S. Kasas (2017). **Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.** Clin Microbiol Infect.

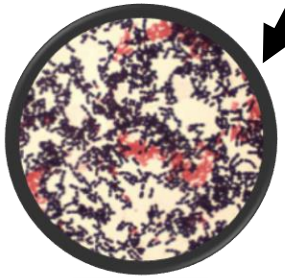
Courtesy Dr Onya Opota / Resistell

Atomic force microscopy on blood culture pellet

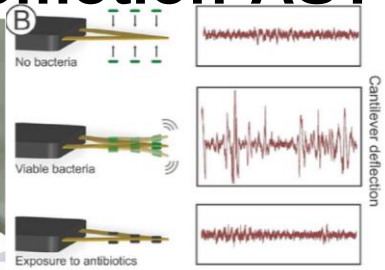
5 ml of positive BC +
40 ml of H2O

CENTRIFUGATION
+
ERYTHROCYTES LYSIS
(Ammonium Chloride)

Bacterial pellet



AFM Nanomotion-AST



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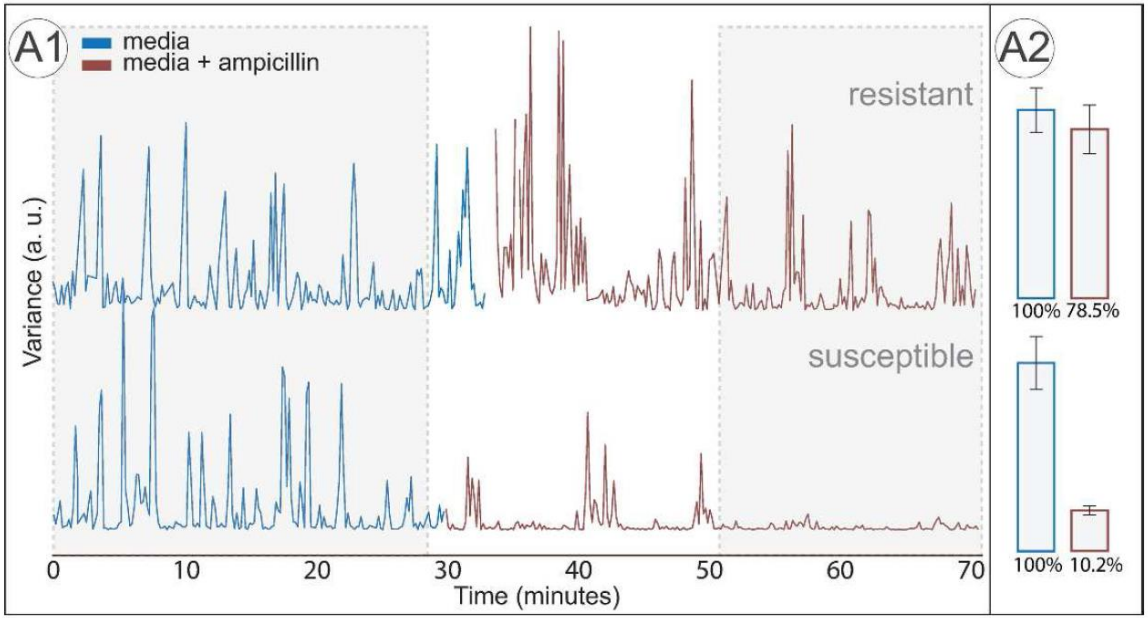
Courtesy Dr Onya Opota

Atomic force microscopy on blood culture pellet



Variance calculation:
Amplitude each 10 sec. of movement, calculation duration 20 min

Elapsed time from the positive blood culture to the end of the susceptibility test < 3 hours.

Stupar, P., O. Opota, G. Longo, G. Prod'hom, G. Dietler, G. Greub and S. Kasas (2017). **Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections.** Clin Microbiol Infect.

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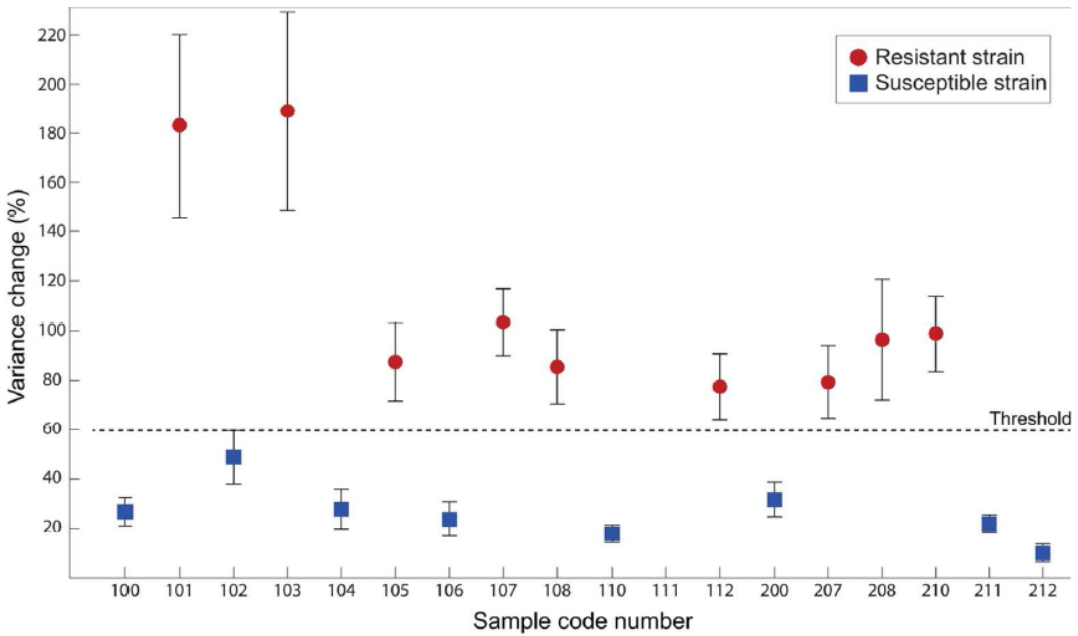

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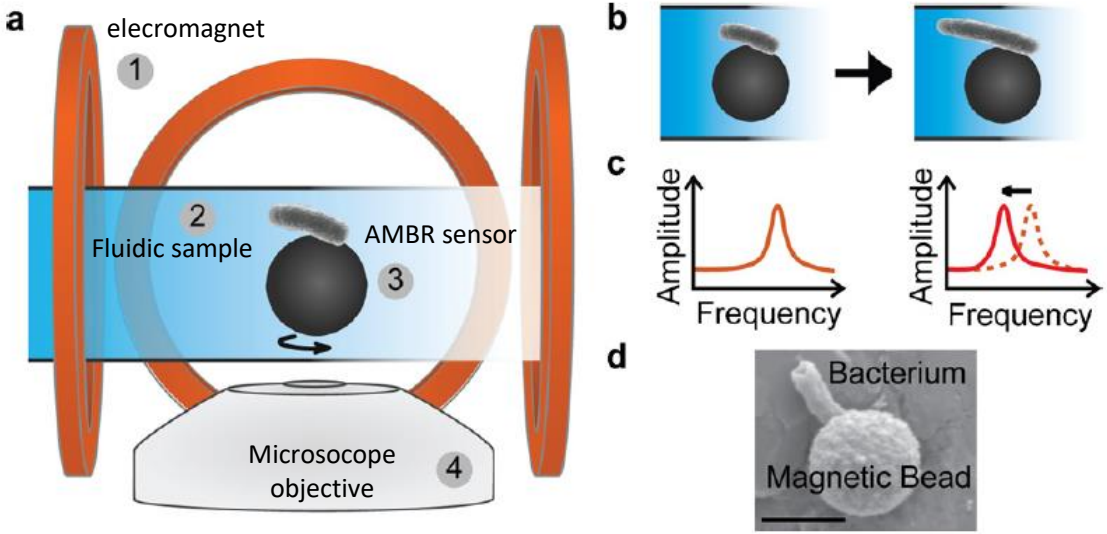
Schumacher et al. *Eur J Clin Microbiol Infect Dis*. 2018
van Belkum et al. *JCM*. 2013

Magnetic bead rotation

asynchronous magnetic bead rotation (AMBR) biosensors



Rotational period change

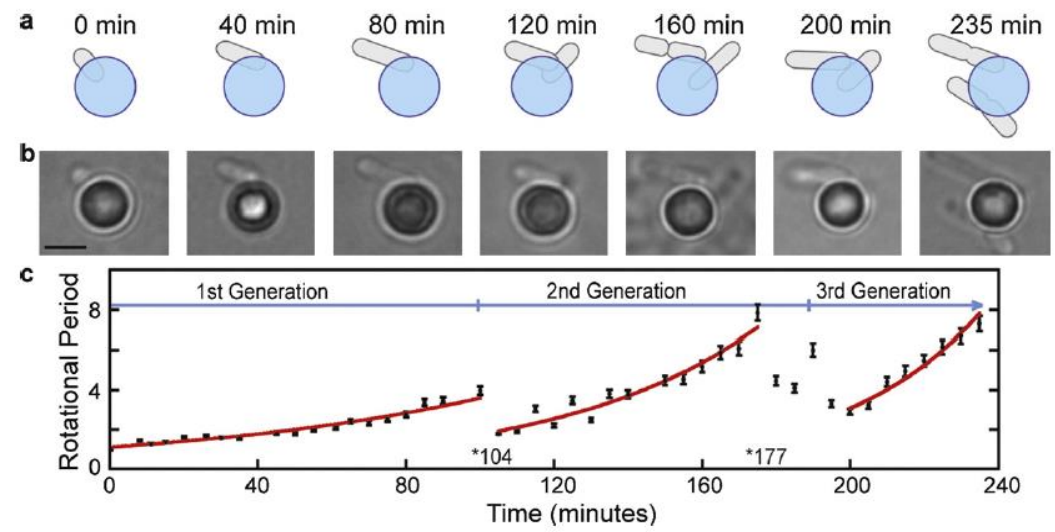
Fig. 1. The concept of measuring single cell elongation using the asynchronous magnetic bead rotation (AMBR) method. (a) A schematic representation of the AMBR sensor on a microscope, where 1. denotes the electromagnet coils, 2. the fluidic sample, 3. the AMBR biosensor with an *E. coli* attached, and 4. the microscope objective. (b) Cell elongation (schematic). (c) Schematic illustrating how the rotational period change is observed as a peak shift in the FFT spectrum (i.e. the elongation of the attached bacterium can be measured by observing the change in the rotational period of the sensor-bacterium complex, which is caused by the increase in the system's effective volume). (d) Scanning electron microscopy image of a single *E. coli* cell attached to a 2.8 μm magnetic bead. The scale bar is 2 μm.

https://www.youtube.com/watch?v=5QSybCpKaBg

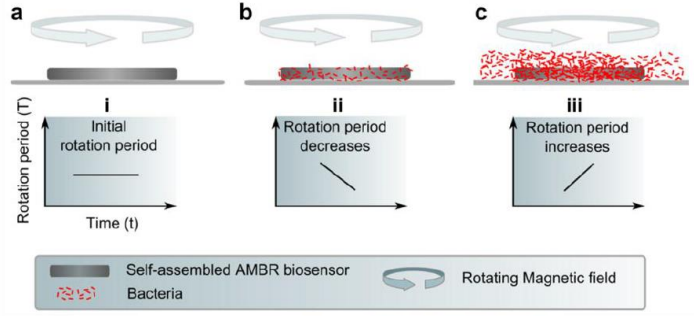
https://www.youtube.com/watch?v=bIKRjfK5P10

P. Kinnunen et al. *Biosensors and Bioelectronics.* 2011

Approach 1 (attached cells)



Approach 2 (unattached cells → Viscometer)



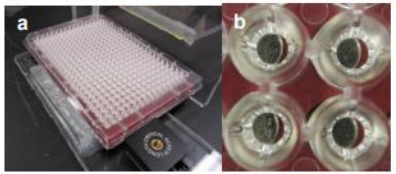
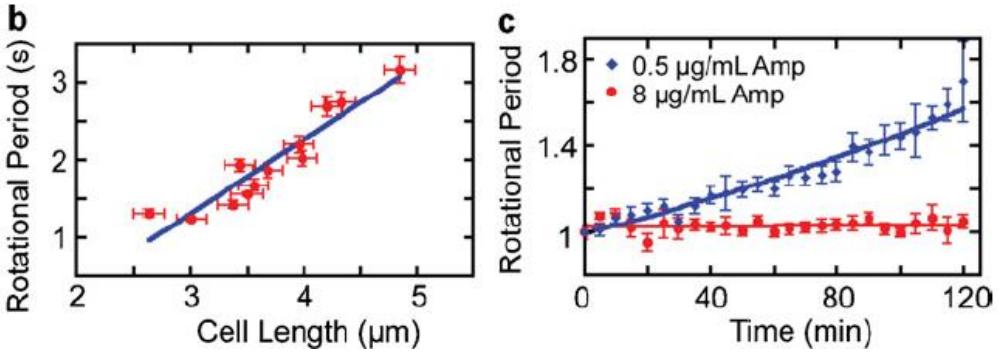
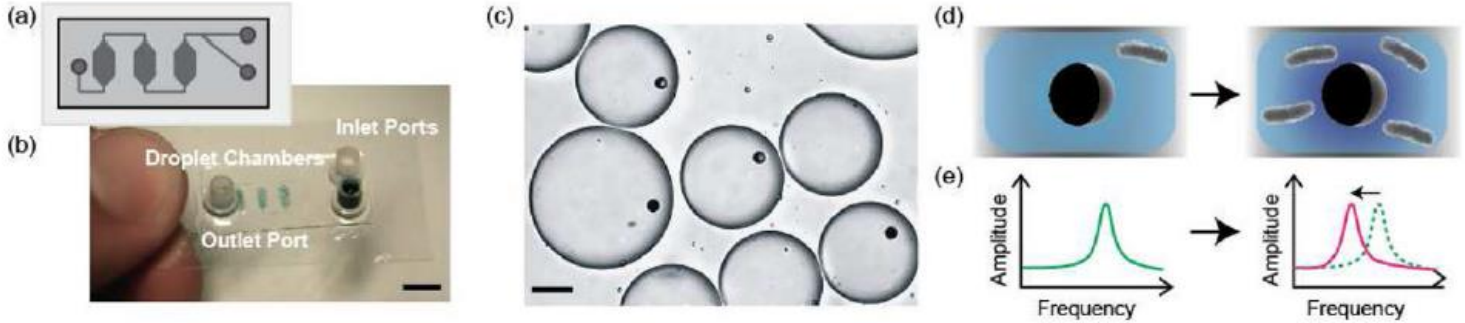


Image of a 384-microwell plate on the magnet array while forming self-assembled AMBR



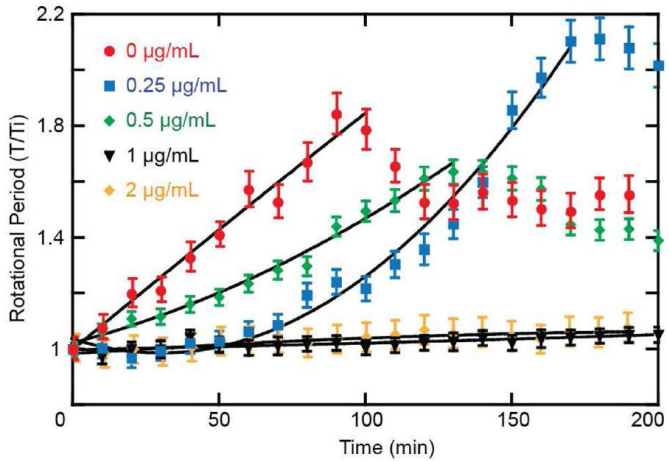
P. Kinnunen et al. / Biosensors and Bioelectronics *26 (2011) 2751–2755*

AMBR micro-viscometer platform for cell proliferation studies.



Rotational period change

- Bacterial growth in the surrounding solution
- Changes the solution viscosity → shifts the particle's rotational frequency.



AMBR viscometer response to the proliferation of *E. coli* exposed to gentamicin
MIC = 1 µg/ml

Sinn et al. *Anal Chem*. 2012

+

- Very fast: ~ 1.5h
- Very high sensitivity (1 cell)
- High throughput potential, miniaturization, multiple antibiotics:
  - 384-microwell plates
  - microfluidic droplet device (nanoliter compartments water in oil droplets containing 50 or fewer bacterial cells /droplets)

-

POP only

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Schumacher et al. *Eur J Clin Microbiol Infect Dis*. 2018
van Belkum et al. *JCM*. 2013

https://youtu.be/AENnU0vdjPo

Microfluidics and microdroplets methods

- Enable the building of **complex in vitro systems on one chip**
- **Flexibility**
- **Temporal and spatial control** over fluids and physical parameters
- Great potential for **reduction in workload and materials**
- High **reproducibility**
- Very **high sensitivity** (single cells)
- Integration of **sensors** to obtain direct and in situ read-out
  - electrochemical
  - optical (fluorescence)
  - microcalorimetric

Jiang et al. *Eur J Clin Microbiol Infect Dis*. 2016
Baraban et al. *Lab Chip*. 2011
Boitard et al. *PNAS*. 2012
Boitard et al. *Eng Life Sci*. 2015
Schumacher et al. *Eur J Clin Microbiol Infect Dis*. 2018
Baltekin et al. PNAS. 2017

Pubmed

Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging

Cell traps

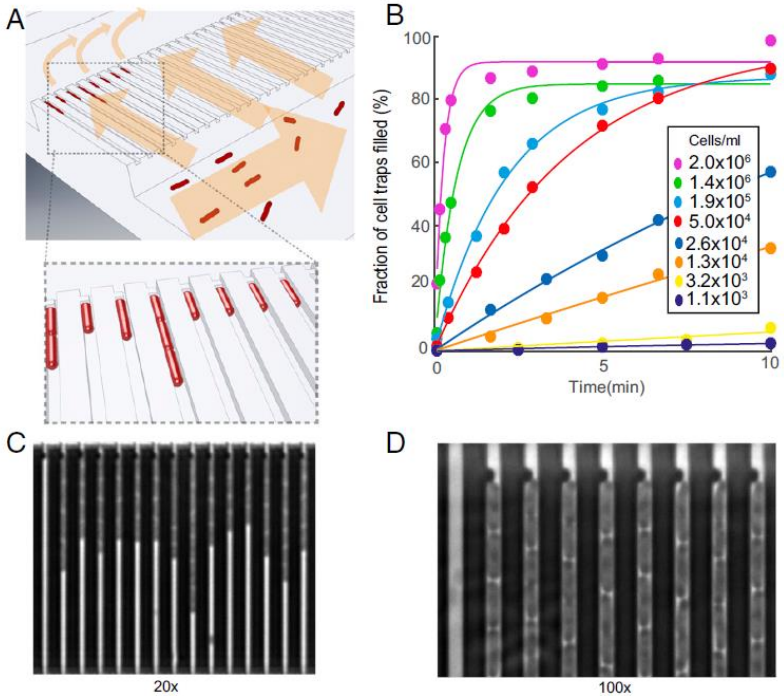

Fig. 1. Design and operation details of the microfluidic chip. (*A*) Cartoon illustrating the loading of rod-shaped bacterial cells (red) into cell traps. Arrows indicate flow direction during loading. (*B*) Fraction of cell traps with at least one *E. coli* cell at different time points. The different markers correspond to different density cell cultures. (*C*) A phase contrast image of *E. coli* in the microfluidic device (darker regions) using a 20× objective. (*D*) A small part of a phase contrast image taken at 100× showing the back end of the cell trap, where the flow restriction region captures the cells during loading.

Baltekin et al. PNAS. 2017

https://astrego.se/technology/

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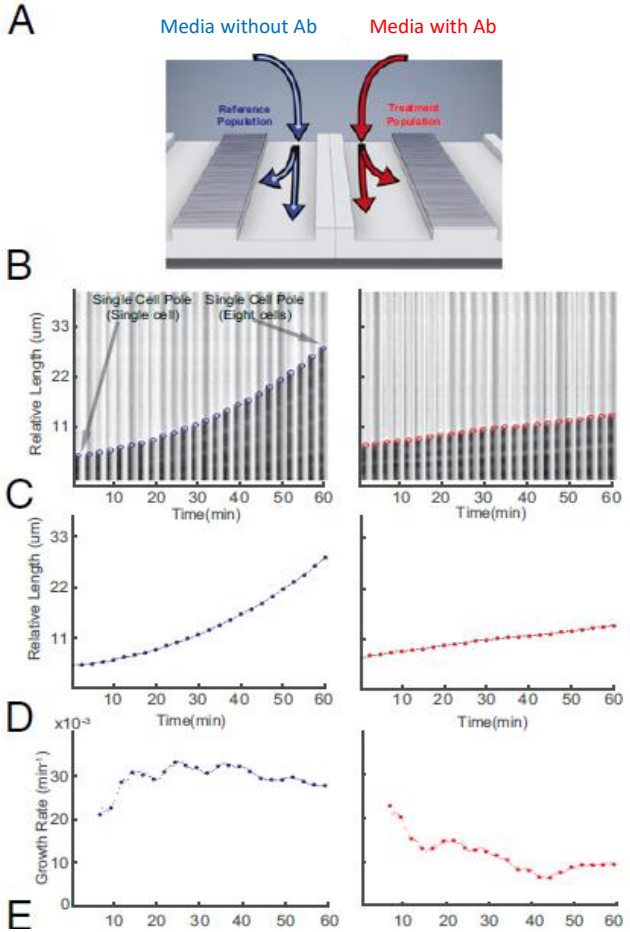
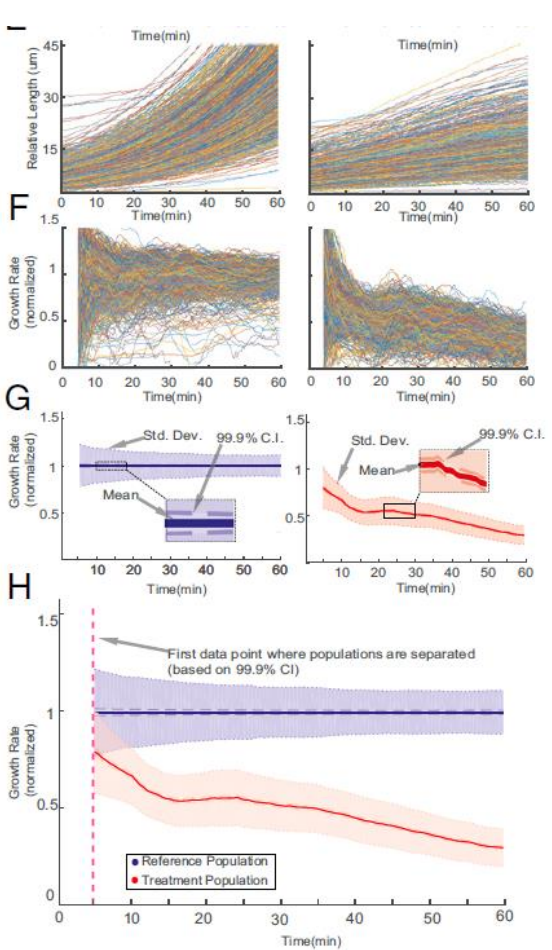
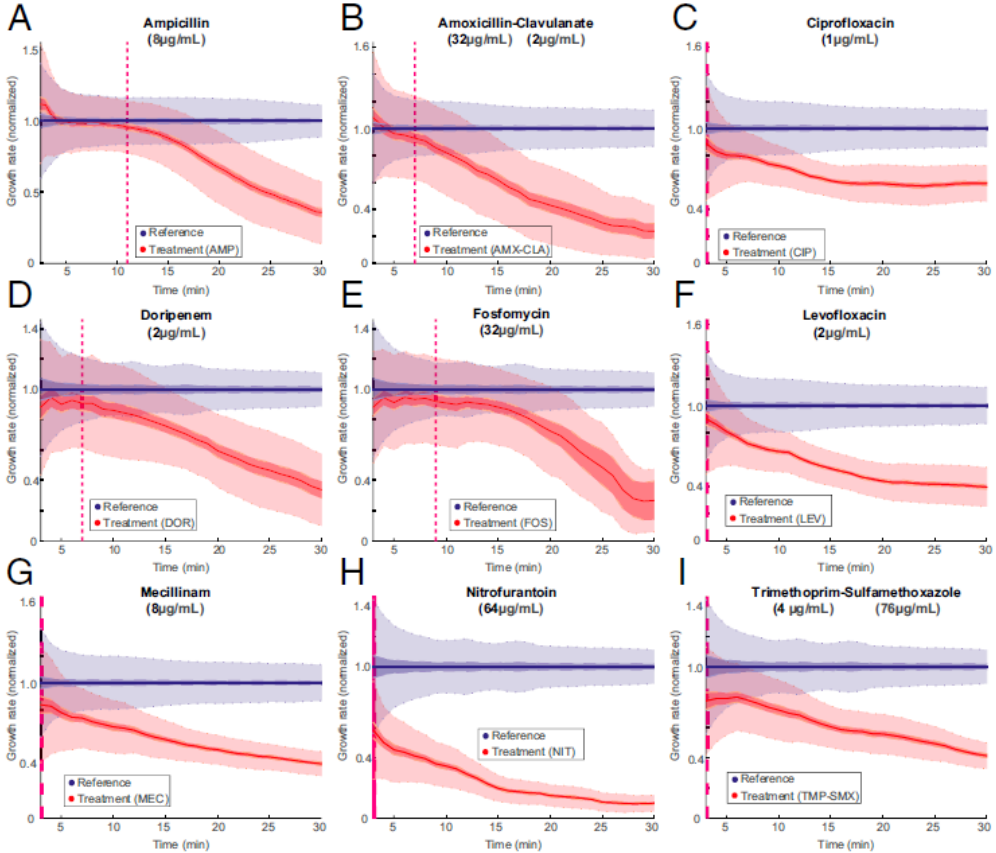
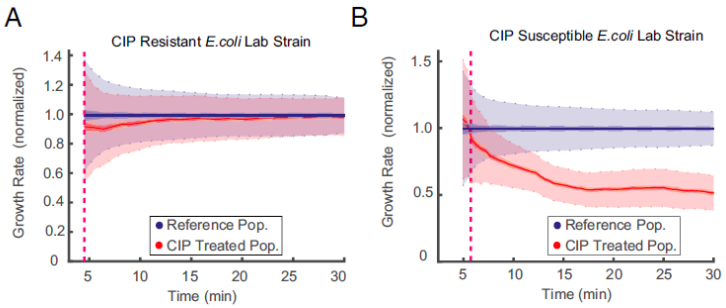




Baltekin et al. PNAS. 2017

Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging





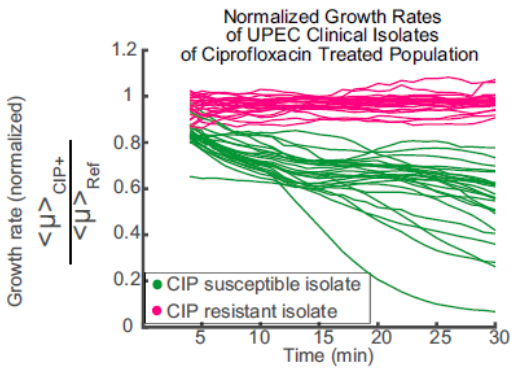


Fig. 3. Fast detection of response to antibiotic treatment. fASTest experiments testing how fast susceptible *E. coli* cells respond to (*A*) ampicillin, (*B*) AMX-CLA, (*C*) CIP, (*D*) DOR, (*E*) FOS, (*F*) LEV, (*G*) MEC, (*H*) NIT, and (*I*) TMP-SMX. AMP, ampicillin.

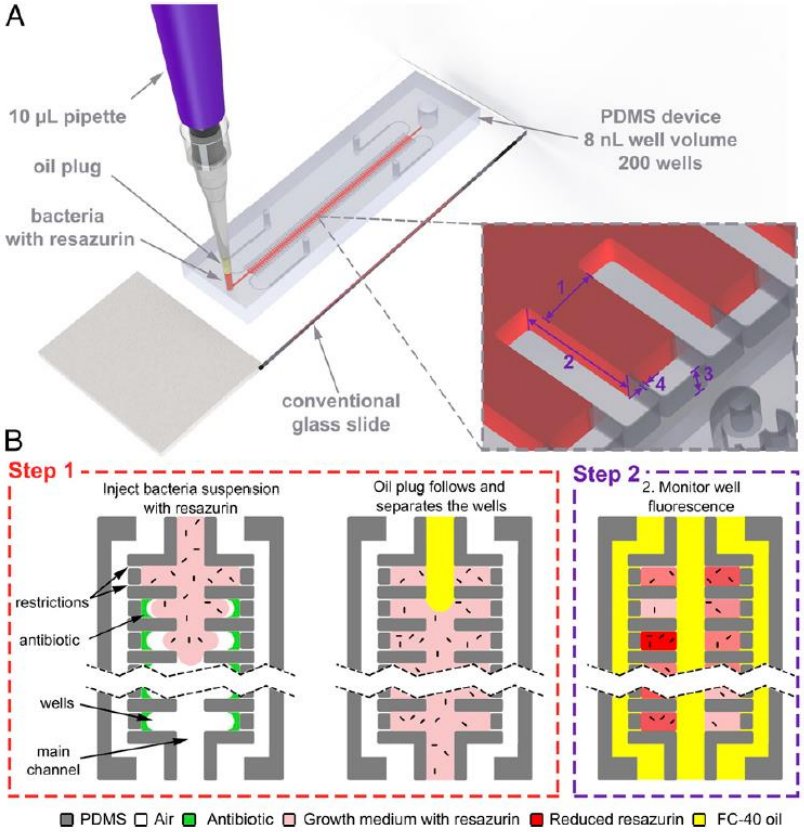
Baltekin et al. PNAS. 2017

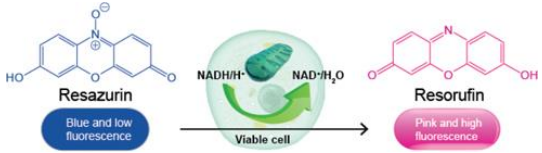
https://astrego.se/products/

https://youtu.be/MHnjd2NjrD0

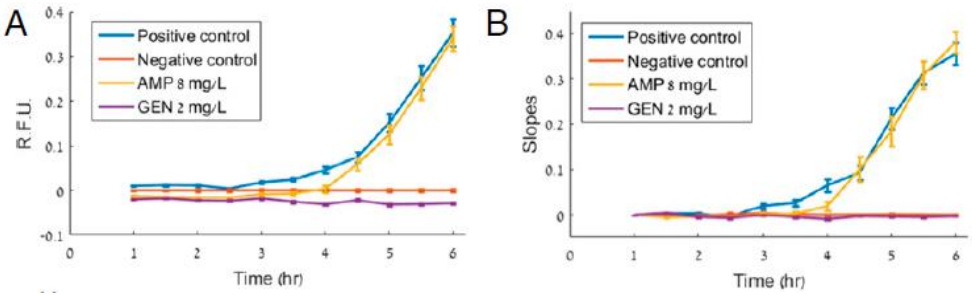
Nanoliter arrays

stationary nanoliter droplet array (SNDA)– AST system



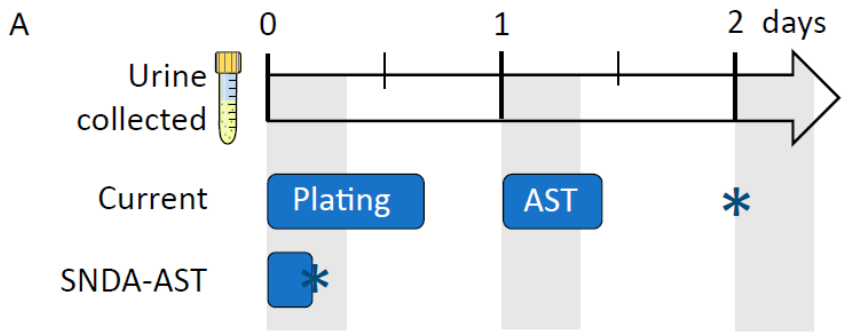


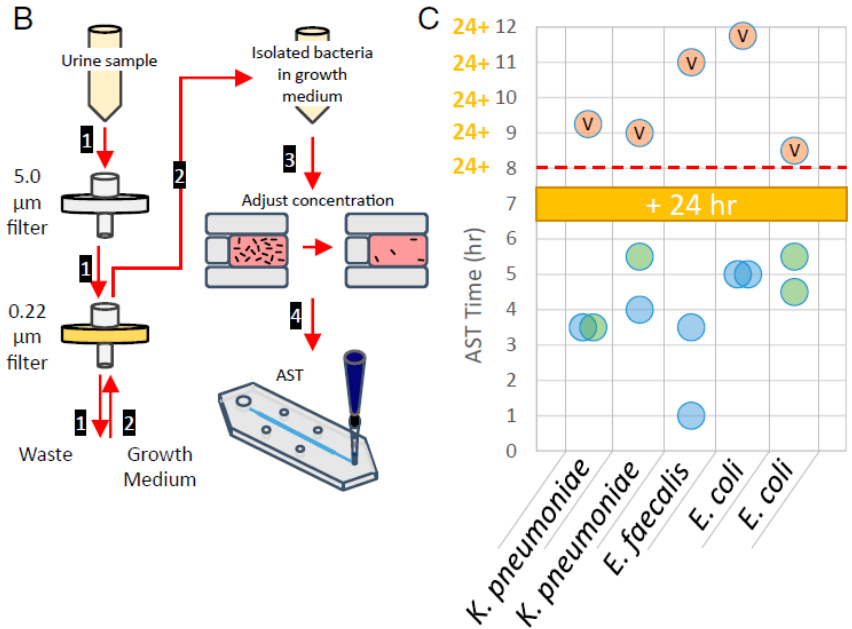
Well fluorescence →proportional to the amount of bacteria/metabolism in the well.



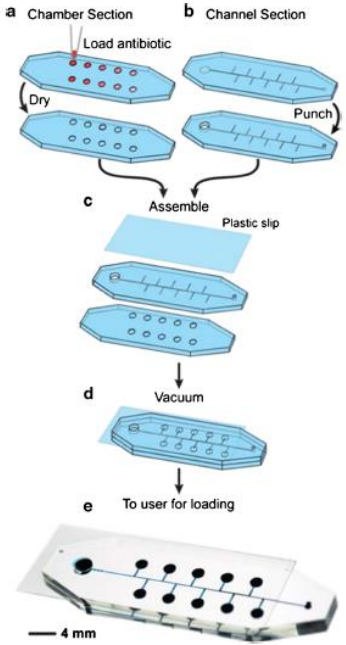
AMP/R
GEN/S

Avesar, J et al. PNAS. 2017





Fabrication and assembly of a self-loading microfluidics device



Avesar, J et al. *PNAS*. 2017 / Schumacher et al. *Eur J Clin Microbiol Infect Dis*. 2018

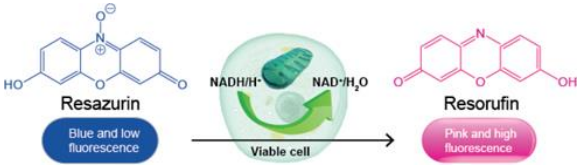
Microdroplets

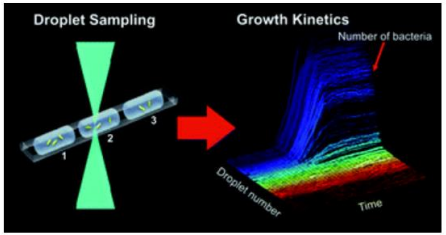
What is droplet-based microfluidics?

https://www.elveflow.com/microfluidic-reviews/general-microfluidics/a-general-overview-of-microfluidics/

What is droplet-based microfluidics? - YouTube

Microdroplets





Baraban, L. *Lab on a chip*. 2011

novel millifluidic droplet analyser (MDA) for precisely monitoring the dynamics of microbial populations over multiple generations in numerous (≥10^3) aqueous emulsion droplets (~100 nL).

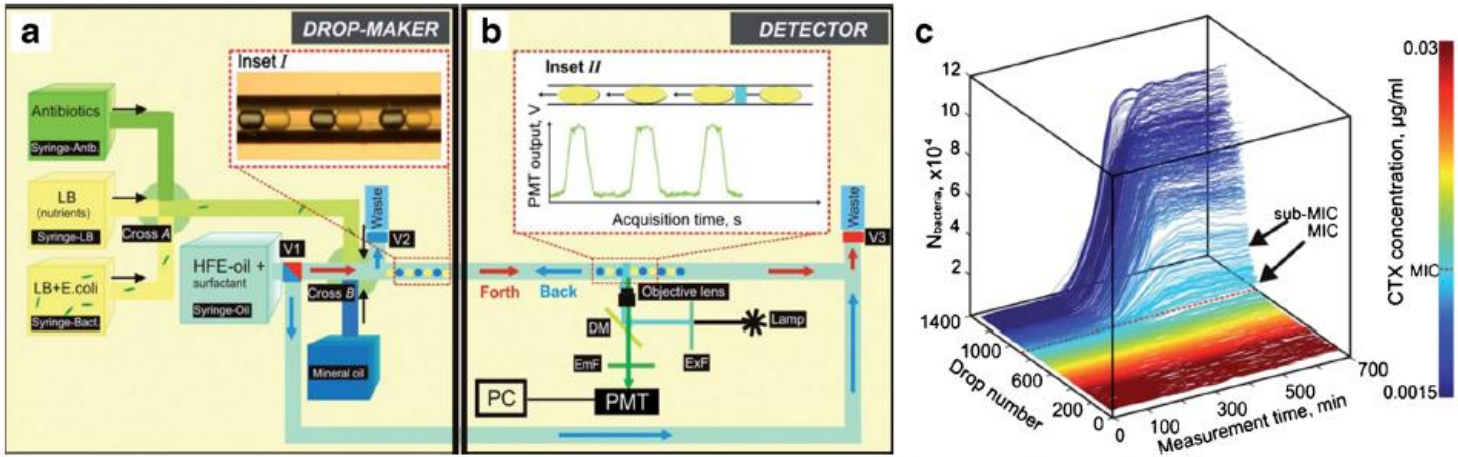
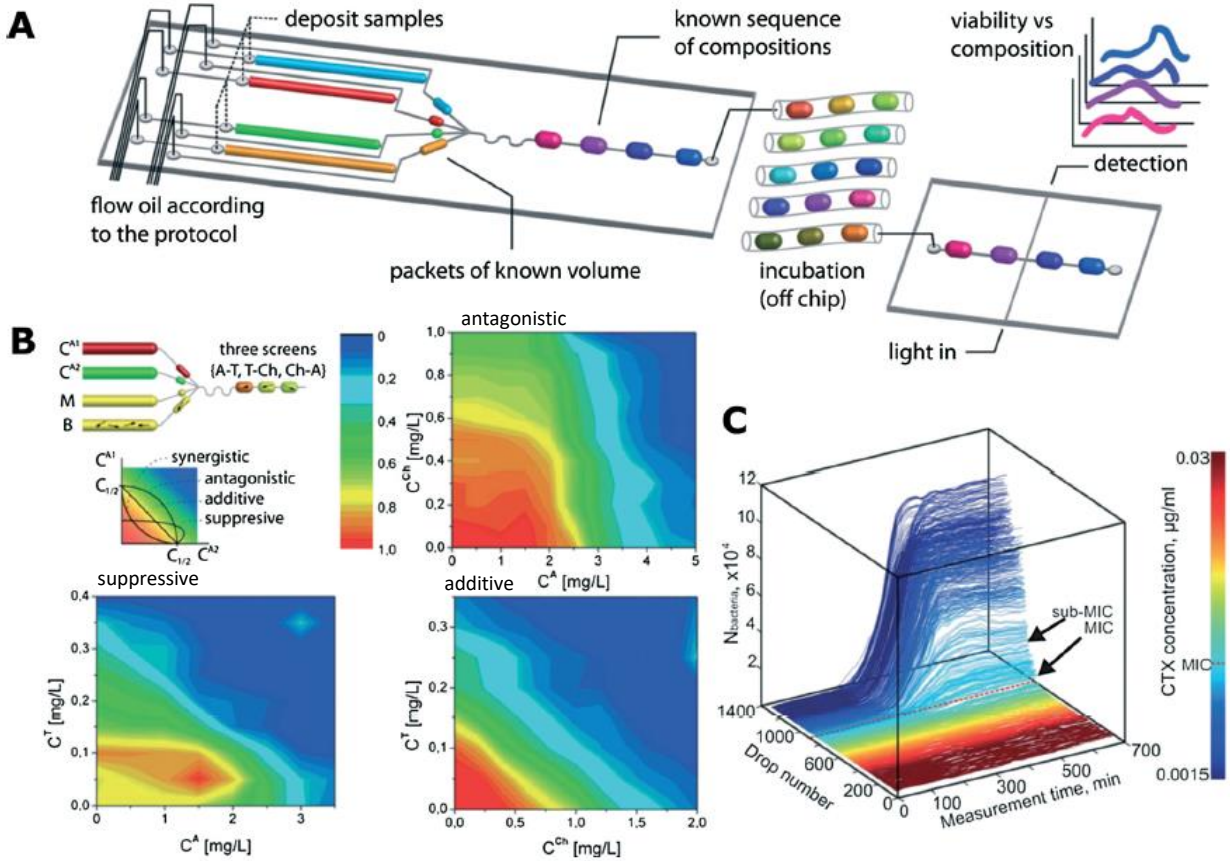


Fig. 10 Schematic representation of the microfluidics droplet analyser, showing the drop maker **a** and detector compartment **b**, which analyses bacterial growth over time. **c** The bacterial growth is plotted for each antibiotic concentration (colour range) to determine the MIC. HFE = hydrofluoroether (HFE) oil, LB = syringe containing nutrients or bacteria, PMT = photomultiplier tube, DM = dichroic mirror, EmF = emission filter, ExF = excitation filter. Adapted with permission from Baraban et al. [117]. Copyright (2017) Royal Society of Chemistry

Schumacher et al. *Eur J Clin Microbiol Infect Dis*. 2018

Antibiotic susceptibility studies using trains of droplets in microfluidic channels



Kaminski et al. Lab chip.2016

Churski et al. Lab chip.2012

Microdroplets of controlled and predefined volume and composition (media, bacteria, reagents, fluorescence substrate resazurin) are generated and incubated in long microtubing as droplet trains

After the incubation, the fluorescence of droplets is measured

Conclusions

Which technology(ies) is/are fulfilling our (future) needs?

Microfluidics

Needs/requirements	Rapid PCR	Rapid IC	Genomics	MALDI	Single cell Imaging	FC	Light scat	Cantilever	Magnetic rotation	Microdroplets	Nanoliter array
High performance (EA, CA, reproducibility, repeatability,..)	?????										
High sensitivity (low bacterial loads)											
Directly from sample											
MIC											
Large panel of Antibiotics											
Direct detection of resistance mechanisms											
Rapid											
High throughput											
Automated (minimal hands-on time)											
Miniaturised (nanotechnology)											
Low cost (high cost ? patient selection)											
N° Y											

- Laboratory impact?
- Clinical impact?
- Added value?

Conclusions

Which technology(ies) is/are fulfilling our (future) needs?

								Microfluidics			
Needs/requirements	**Rapid PCR**	**Rapid IC**	**Genomics**	**MALDI**	**Single cell imaging**	**FC**	**Light scat**	**Cantilever**	**Magnetic rotation**	**Microdroplets**	**Nanoliter array**
High performance (EA, CA, reproducibility, repeatability,..)	Y	Y	Y	Y	Y	Y	Y	?	?	?	?
High sensitivity (low bacterial loads)	Y	N	V	Y	N	Y	Y	Y	Y	Y	Y
Directly from sample	Y	Y	Y	M	N	N	N	N	?	Y	Y
MIC	N	N	N	N	Y	Y	N	Y	Y	Y	Y
Large panel of Antibiotics	N	N	N	N	Y	Y	Y	V	Y	Y	Y
Direct detection of resistance mechanisms	Y	Y	Y	Y	N	N	N	N	N	N	N
Rapid	Y	Y	N	V	M	Y	Y	Y	Y	Y	Y
High throughput	Y	N	N	N	N	M	Y	?	Y	Y	Y
Automated (minimal hands-on time)	Y	N	Y	N	Y	Y	Y	?	Y	Y	Y
Miniaturised (nanotechnology)	V	Y	Y	N	N	N	N	?	Y	Y	Y
Low cost (high cost ? patient selection)	V	Y	N	Y	N	?	?	?	?	?	?
N° Y	**7**	**6**	**5**	**4**	**4**	**6**	**6**	**3**	**7**	**8**	**8**

Pubmed	**N° hits**
cantilever antibiotic	17
nanomechanical sensor antibiotic	3
microfluidic antibiotic	346
magnetic rotation antibiotic	125

- Laboratory impact?
- Clinical impact?
- Added value?

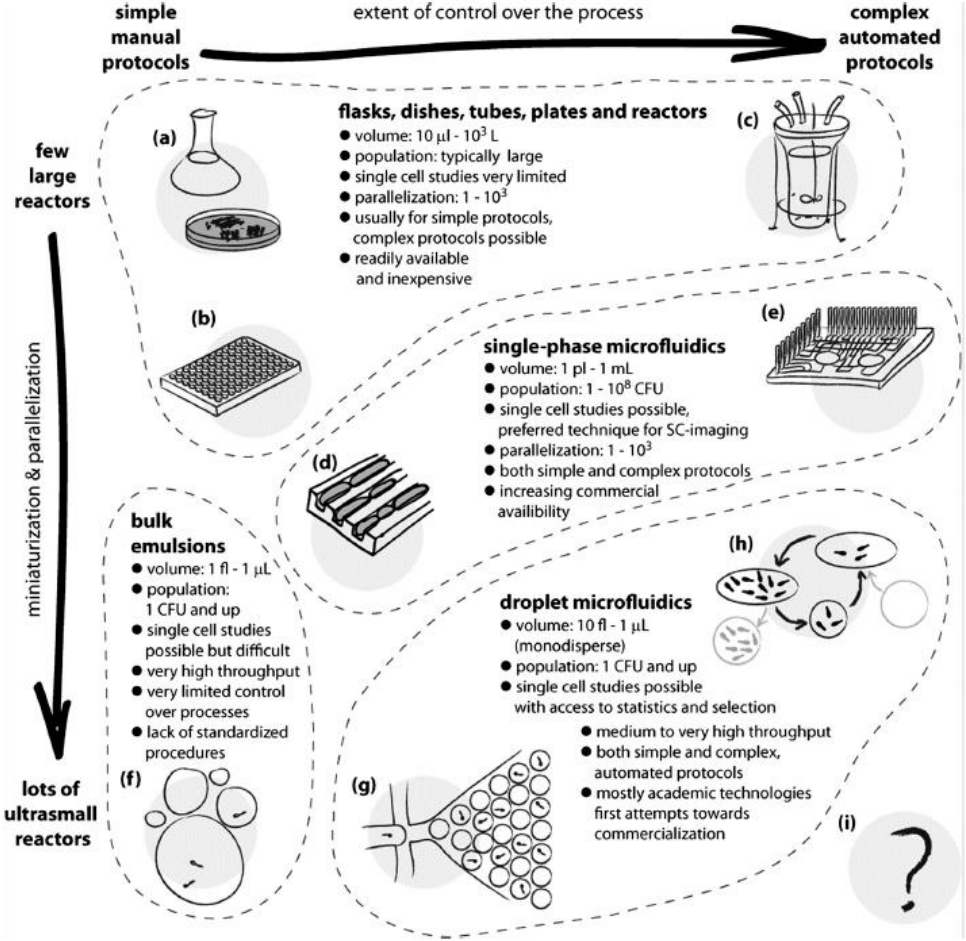
Y: Yes
N: No
V: Variable
M: Moderate
?: Unknown

+

- Fast: 30min -6 hrs
- Very high sensitivity (1 cell)
- High throughput potential
- Miniaturization
- Multiple antibiotics tested/run
- Antibiotics combinations (synergy, antagonism,..)

-

POP only



Kaminski, T.S et al. Lab chip.2016

Conclusions

Study groups to provide recommendation and decision criteria to choose the right technologies that are adapted to laboratory and patients needs

http://hands-of-peace.org/7-benefits-of-study-groups/