Geçen Yılın Ezber Bozan Makaleleri

“Tanı”

Onur KARATUNA
Acıbadem Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilim Dalı
İstanbul

EUCAST Development Laboratory
Clinical Microbiology
Växjö, İsveç

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Makale Seçim Ölçütleri

- Son 12 ay (Nisan 2017 – Mart 2018)
- PubMed kaynaklı
- “Diagnostics”, “microbiology”, “infectious diseases” anahtar sözcükleri ile listelenen İngilizce makaleler arasından
- Subjektif

IDSA
Better Tests, Better Care: Improved Diagnostics for Infectious Diseases

- Tanı teknolojilerindeki gelişmelere rağmen aşağıdaki özellikte testlere acil ihtiyaç bulunuyor
    - kullanımı kolay
    - enfeksiyon etkeni mikroorganizmayı tanımlayan
    - ilaçlara direncini belirleyen
    - mevcut testlerden daha hızlı sonuç sunan

Biyoteknoloji Alanındaki Gelişmeler

- 3 boyutlu yazıcı ile
- Düşük maliyetli, taşınabilir, kantitatif PCR cihazı
- 12 x 7 x 6 cm boyutlarında

Mulberry *et al.* (2017) 3D printing and milling a real-time PCR device for infectious disease diagnostics. PLoS ONE.

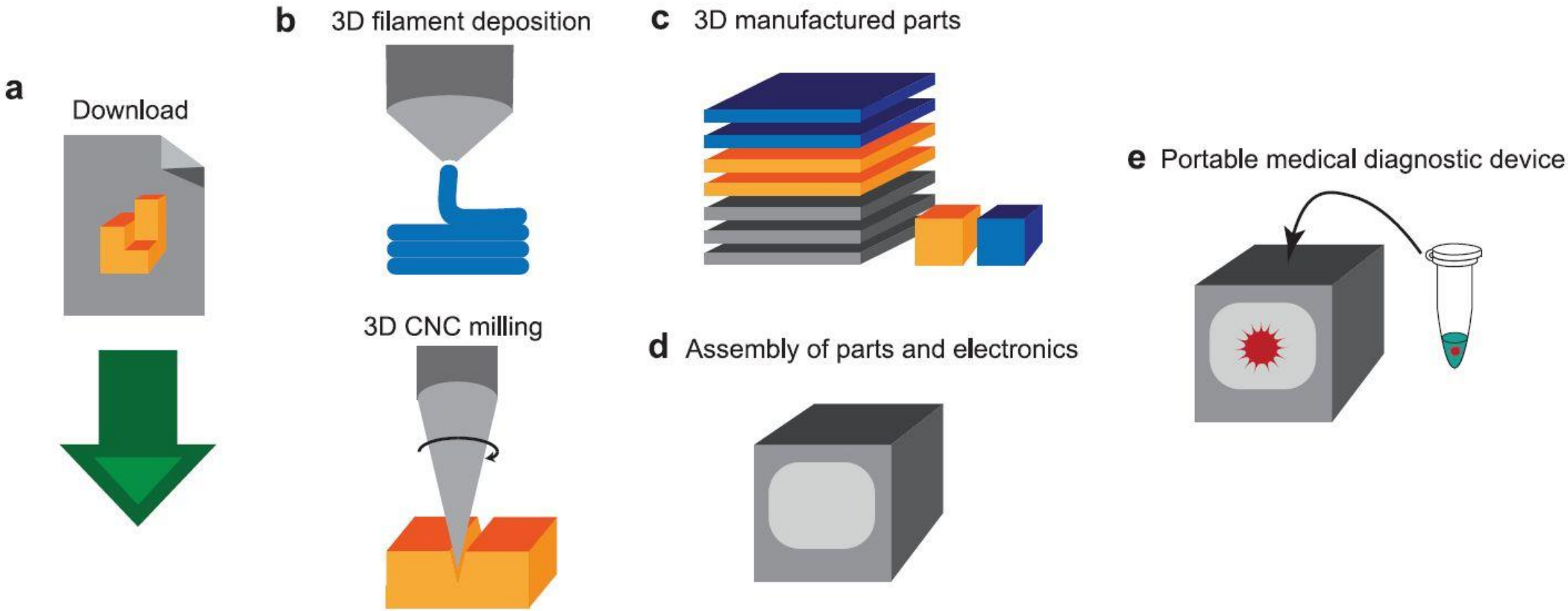


Fig 1. From digital design file to portable diagnostics device. (a) A digital format of design file is uploaded to the internet, and downloaded by users. (b) A user uploads the digital files into 3D printer and 3D CNC milling machine for manufacturing. The 3D printer extrudes and deposits filament and the 3D CNC mill cuts and drills through material to engrave patterns and shapes. (c) This results in 3D manufactured parts for assembly. (d) The parts and off-the-shelf electronics components are assembled. (e) This assembled device can function as a portable medical diagnostic device for detecting and quantifying the pathogen in a sample through qPCR.

https://doi.org/10.1371/journal.pone.0179133.g001

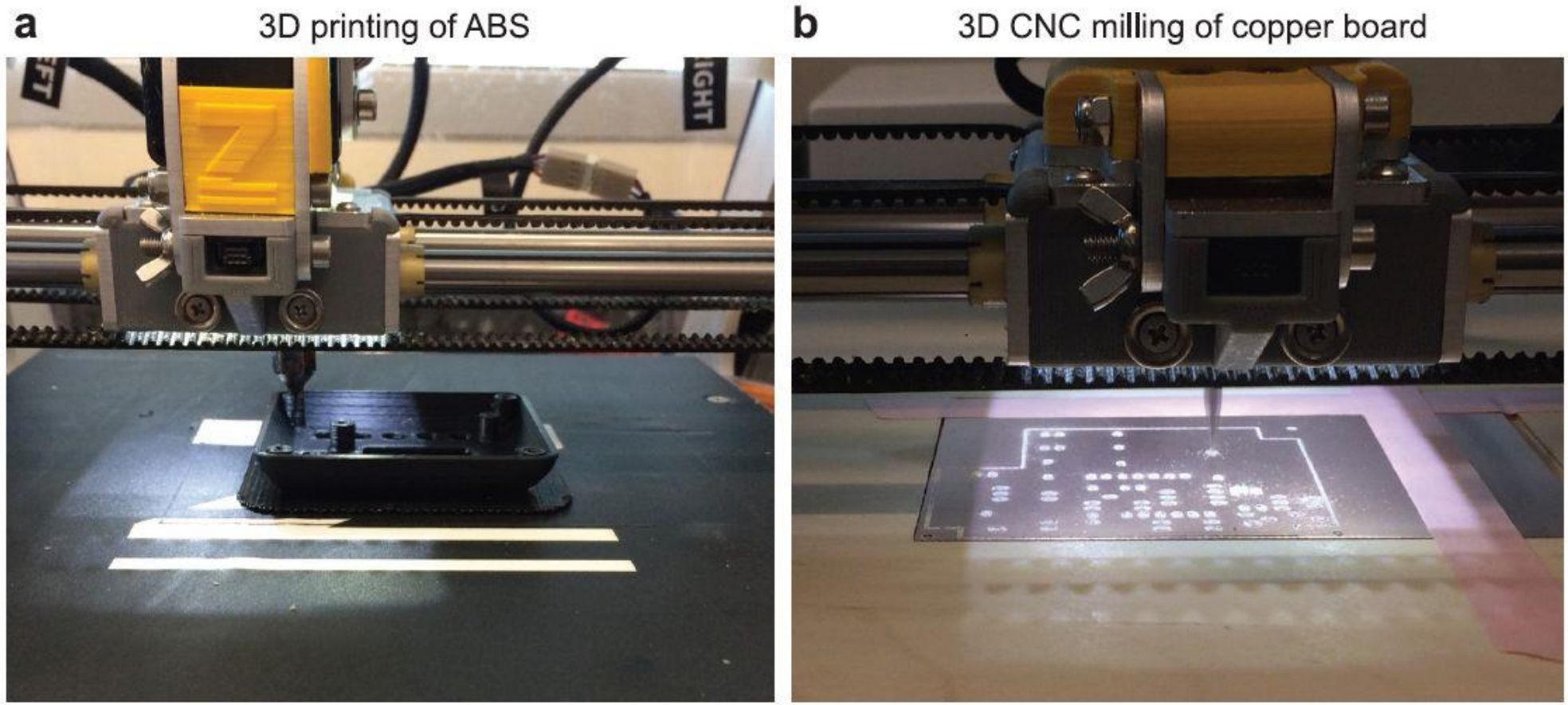


Fig 4. Photographs of 3D printing and 3D milling of all the parts using a multitool 3D printer. (a) The faceplate is being printed with black ABS material on a heated bed. (b) The main board is being engraved using 3D CNC mill.

https://doi.org/10.1371/journal.pone.0179133.g004

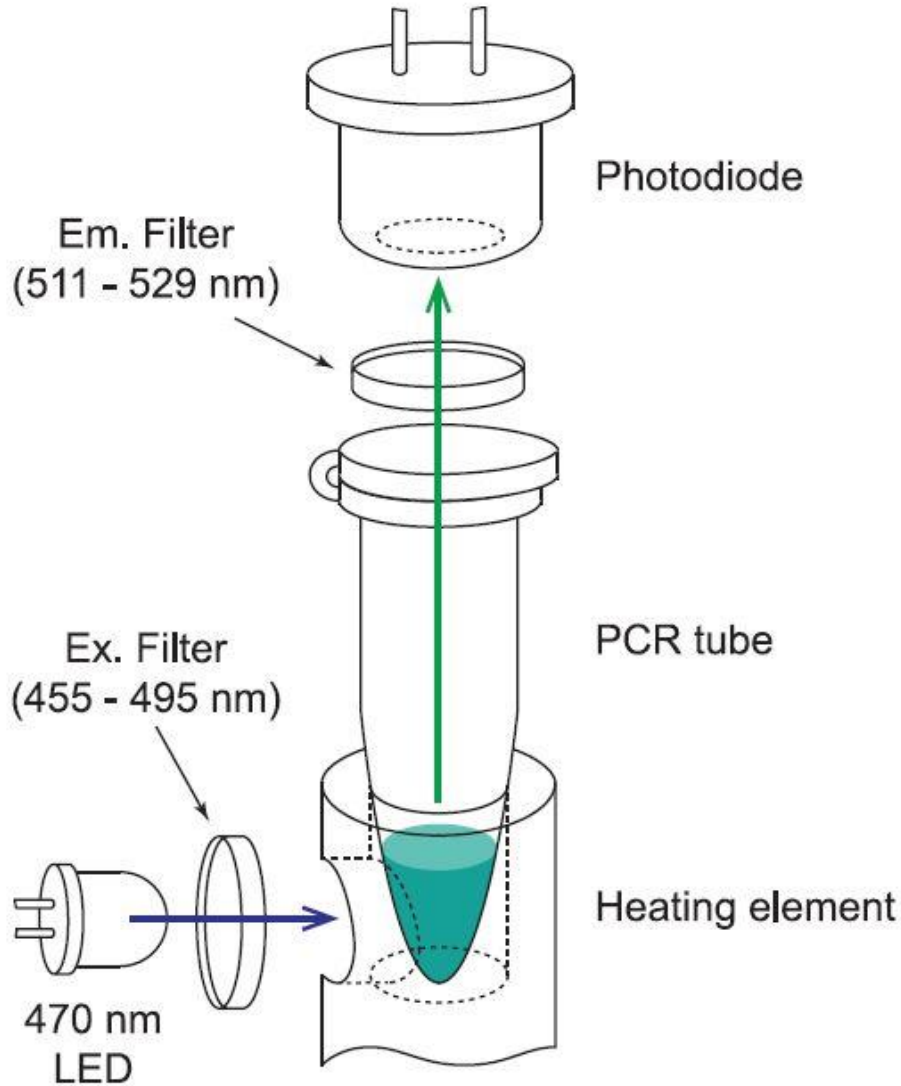


Fig 3. Optical design of real-time fluorescence measurement for qPCR. The blue LED (470 nm) emits excitation wavelength that is filtered through an excitation filter which is a band-pass filter of 455 to 495 nm. The excitation light travels through the hole in the aluminum rod and is absorbed to the sample in the PCR tube. The emitted light is collected by an avalanche photodiode through an emission filter, with a band-pass at 511 to 529 nm.

https://doi.org/10.1371/journal.pone.0179133.g003

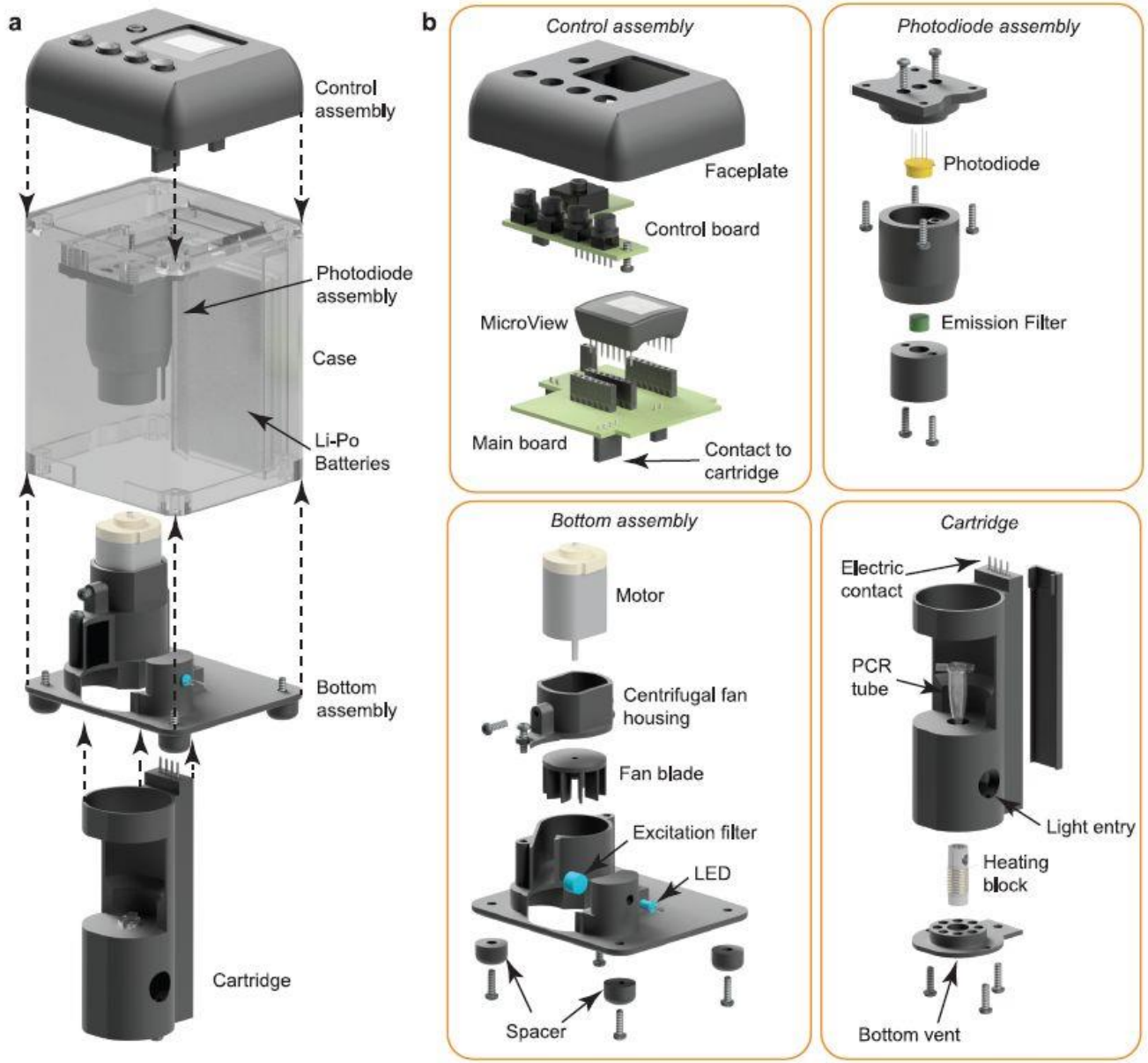


Fig 5. 3D assembly of the system with exploded views. (a) The complete device showing locations of Control assembly, Photodiode assembly, Bottom assembly, Case, and Li-Po batteries. (b) Detail of assemblies. The Control assembly contains a faceplate, a control board, a main board, and a MicroView microcontroller. This assembly provides a user interface and houses all electronic controls. The Photodiode assembly contains the photodiode and emission filter. This assembly is used to determine the target DNA concentration present in the sample. The bottom assembly contains a motor, a fan blade, spacers, and a centrifugal fan housing, forming the cooling fan system. It also contains an LED and excitation filter, establishing the light source for illuminating the sample. The cartridge is a removable assembly allowing for easy insertion of the PCR tube containing the sample. It contains the heating block, electrical contacts for connection to the main board, and a vented bottom plate allowing for the escape of warm air.

https://doi.org/10.1371/journal.pone.0179133.g005

Cihazın toplam maliyeti ≈ 300 USD

Sonuç: Kantitatif real-time PCR

Mulberry *et al.* (2017) 3D printing and milling a real-time PCR device for infectious disease diagnostics. PLoS ONE.

Mikro akışkan temelli platformlar

- Ucuz, tek kullanımlık, tamamen 3D yazıcı ile hazırlanan mikro akışkan reaktör array

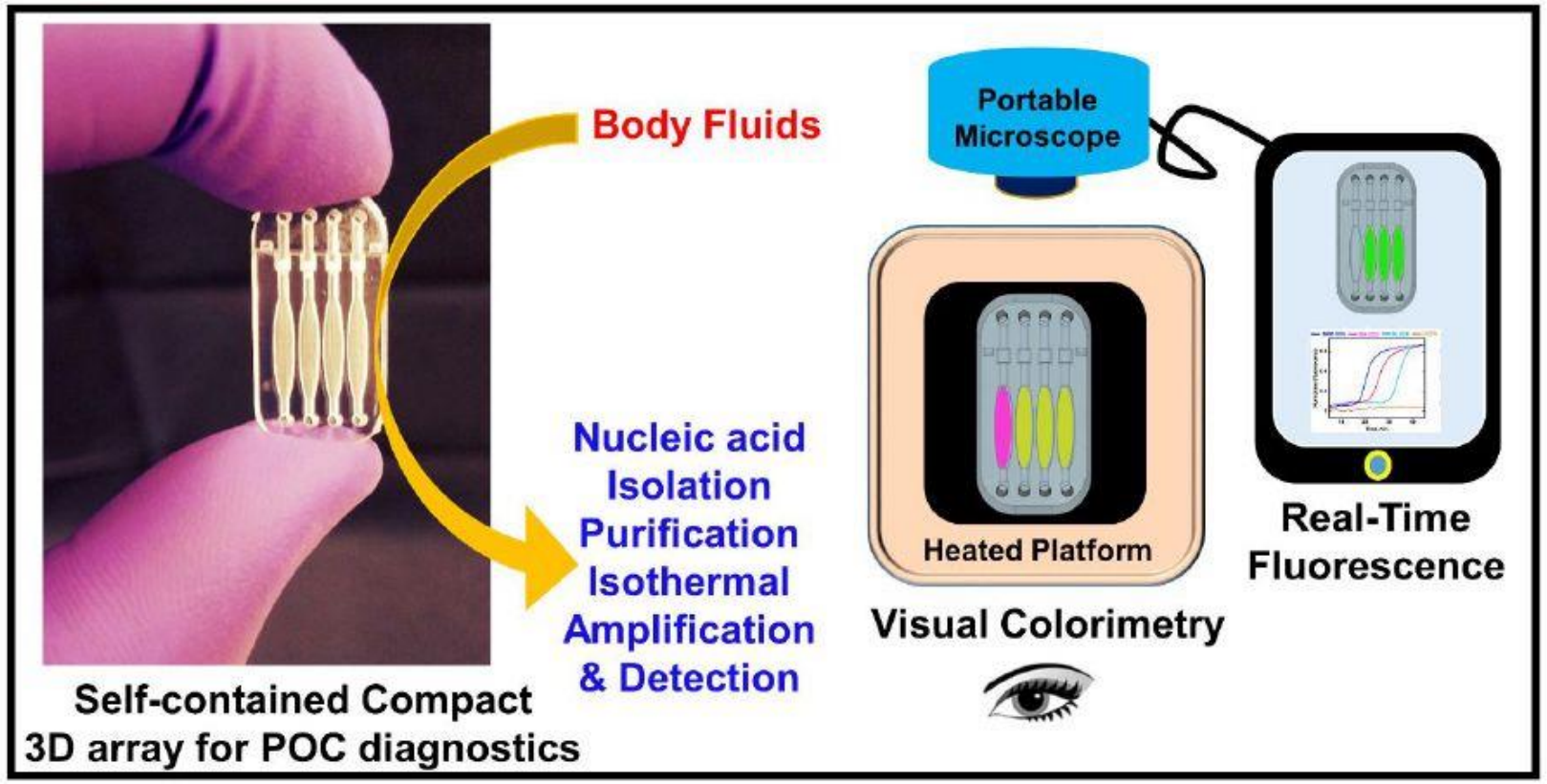
- Nükleik asit ekstraksiyonu ve konsantrasyonu
- İzotermal amplifikasyon
- Kolorimetrik kalitatif tespit
- Real-time floresans ölçümü ile kantitatif tespit

- Enfeksiyon hastalıklarının hasta başı (point of care) tanısı

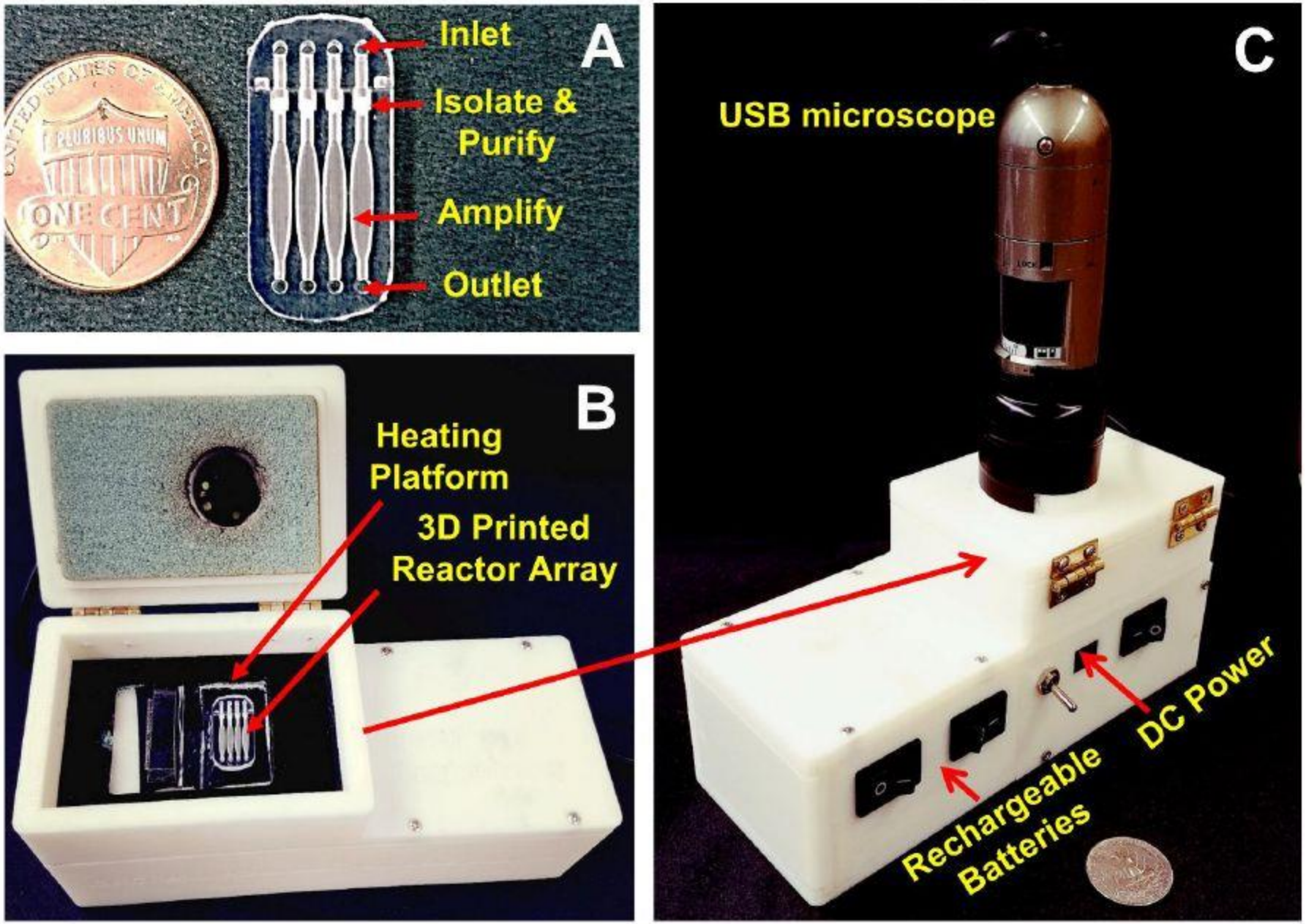
Kadimisetty *et al.* (2018) Fully 3D Printed Integrated Reactor Array for Point-of-Care Molecular Diagnostics. Biosensors and Bioelectric.

- Plazma örneğinde *Plasmodium falciparum*
- BOS örneğinde *Neisseria meningitidis*

- 50 dakikada sonuç



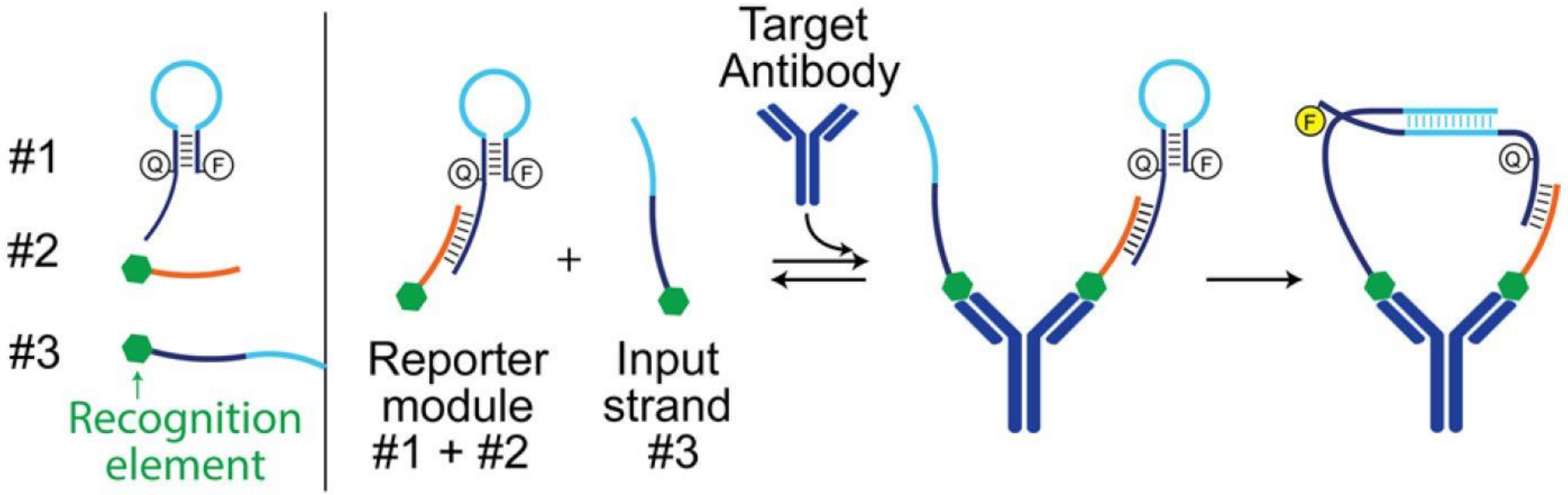
Kadimisetty *et al.* (2018) Fully 3D Printed Integrated Reactor Array for Point-of-Care Molecular Diagnostics. Biosensors and Bioelectric.



Kadimisetty *et al.* (2018) Fully 3D Printed Integrated Reactor Array for Point-of-Care Molecular Diagnostics. Biosensors and Bioelectric.

- Antikor saptanması: tanı ve immün yanıtın takibi

- Antijen nükleik asit zincirine bağlanıyor
- Antikorla bağlandığında sinyal oluşuyor



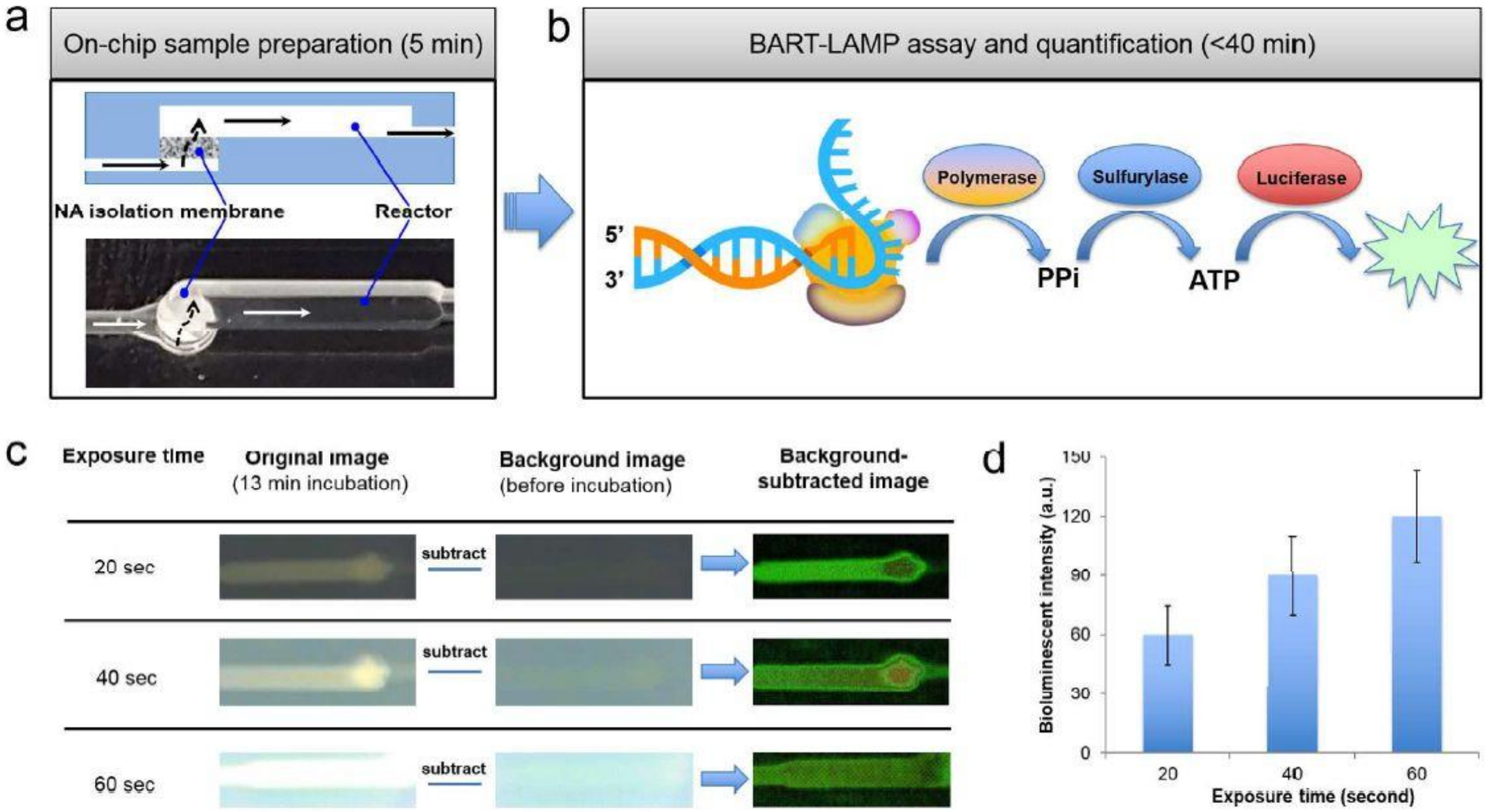
Porchetta *et al.* (2018) Programmable Nucleic Acid Nanoswitches for the Rapid, Single-Step Detection of Antibodies in Bodily Fluids. *J. Am. Chem. Soc.*

- Yüksek duyarlılık
- Mükemmel özgüllük
- Hızlı yanıt süresi (<5 dakika)
- Aynı çözeltide birden fazla antikorun aranabilmesi

- Peptid bazlı AT20 faz-1 terapötik aşı ile aşılanan HIV pozitif hastaların takibinde başarılı

- Örnek başı maliyet 0.01 USD

Porchetta *et al.* (2018) Programmable Nucleic Acid Nanoswitches for the Rapid, Single-Step Detection of Antibodies in Bodily Fluids. *J. Am. Chem. Soc.*



Song *et al.* (2018) Smartphone-based Mobile Detection Platform for Rapid Molecular Diagnostics and Spatiotemporal Disease Mapping. *Anal. Chem.*

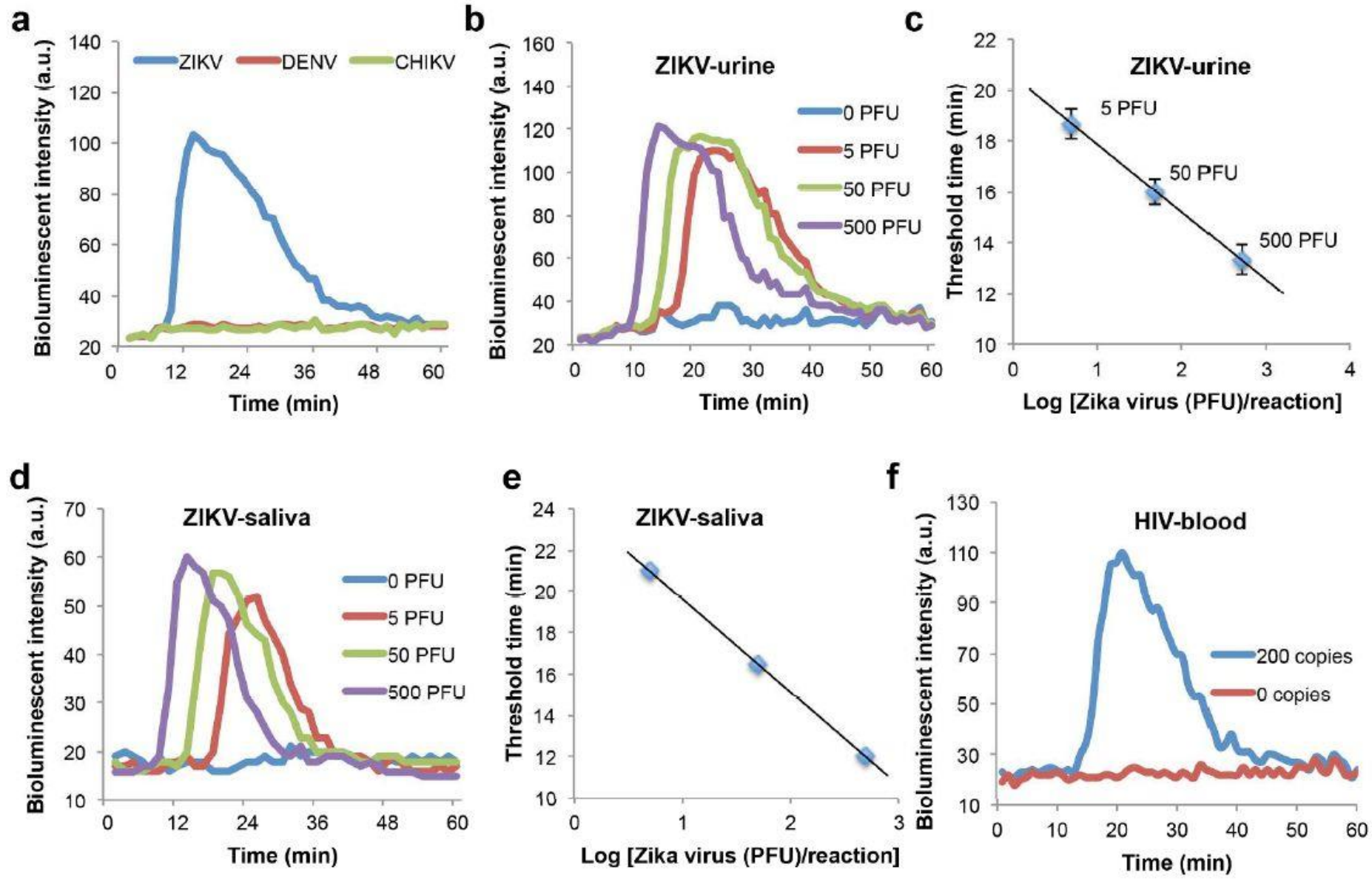
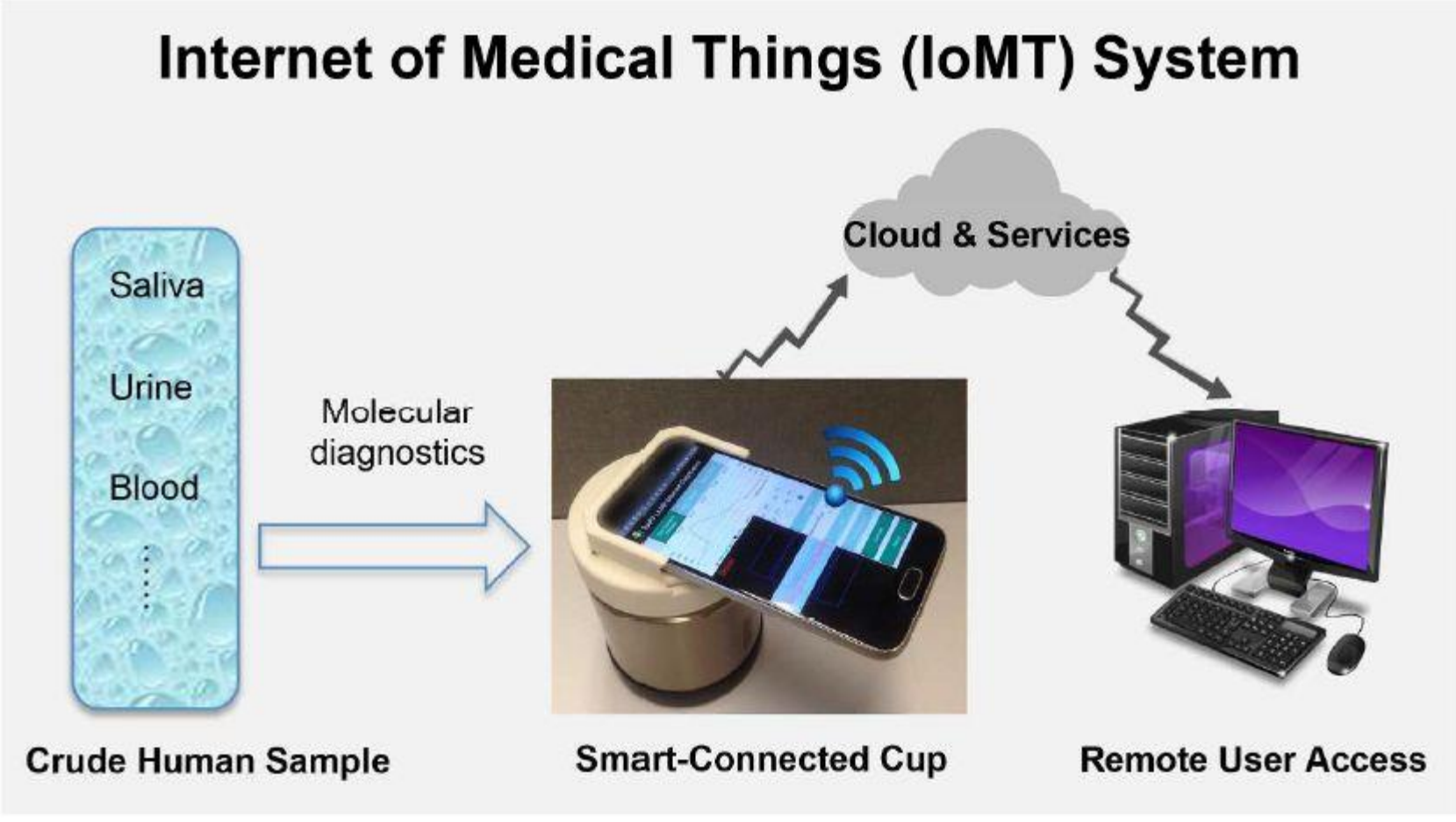


Figure 5. Quantitative detection of ZIKV in urine and saliva, and HIV detection in blood. (a)

Song *et al.* (2018) Smartphone-based Mobile Detection Platform for Rapid Molecular Diagnostics and Spatiotemporal Disease Mapping. *Anal. Chem.*



Song *et al.* (2018) Smartphone-based Mobile Detection Platform for Rapid Molecular Diagnostics and Spatiotemporal Disease Mapping. *Anal. Chem.*

HIV enfeksiyonu tanısında 4. jenerasyon PoC testler

- Alere Determine HIV-1/2 Combo (FDA onayı var)
- Standard Diagnostics BIOLINE HIV Ag/Ab Combo (*SD Combo*)

- Her iki test de HIV-1 ve HIV-2 antikorları ile p24 antijenini serum ve plazmada saptayabiliyor.

- Referans test: 97 pozitif, 36 negatif örnek

	Duyarlılık	Özgüllük	Akut enf. (HIV RNA poz, Ab neg)
• Alere	%95 (%88-98)	%100 (%90-100)	12/13 saptandı
• SD	%91 (%83-96)	%100 (%90-100)	8/13 saptandı

Stafylis & Klausner (2017) Evaluation of two 4th generation point-of-care assays for the detection of Human Immunodeficiency Virus infection. PLoS ONE.

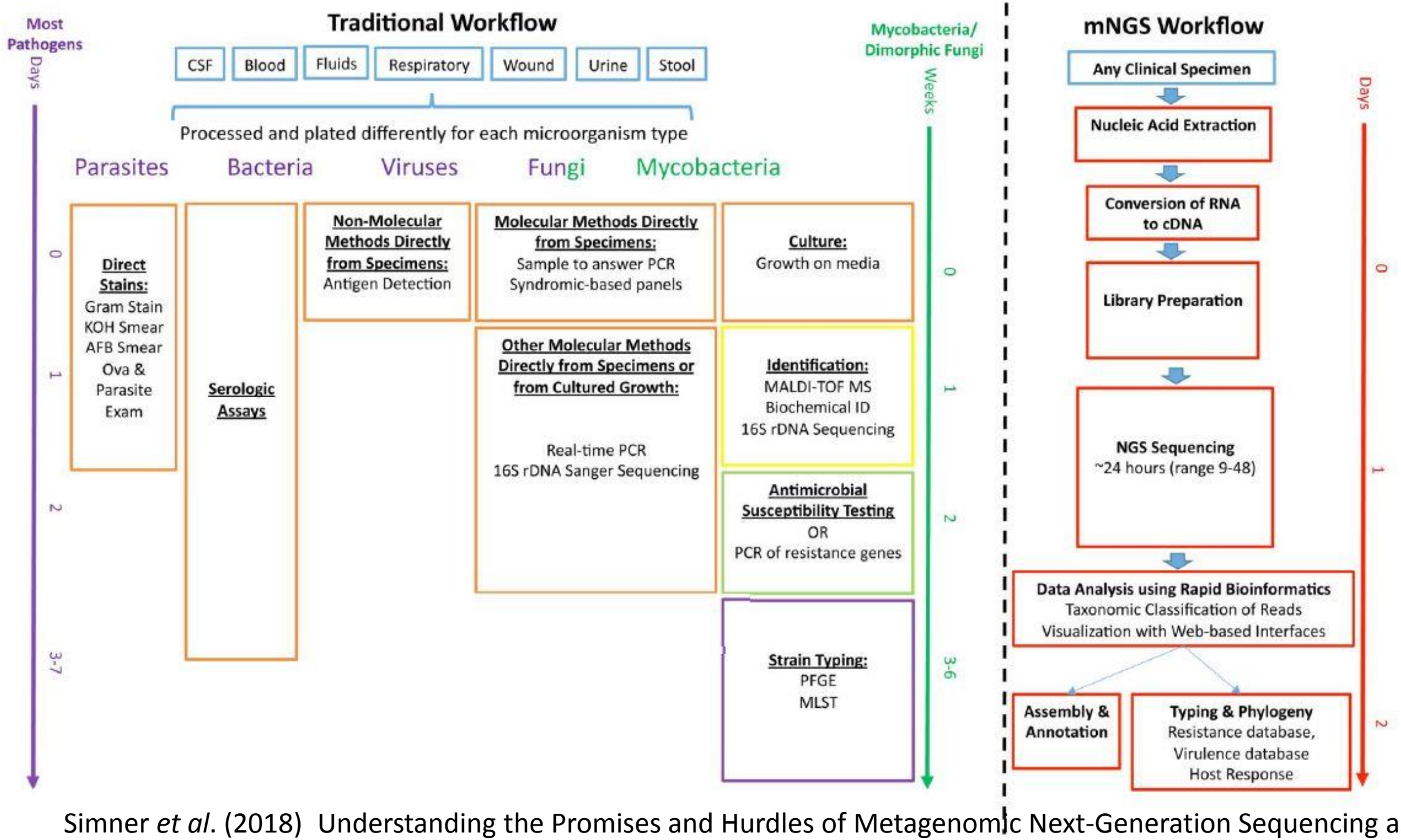
Metagenomik yeni nesil dizileme

- Günümüzde hala enfeksiyon etkenlerinin %60'ı tanımlanamıyor

- Direkt olarak klinik örnekten patojenin tanımlanması
- Bakteri, mantar, parazit, virüs
- Ön bilgiye ihtiyaç yok

Sorunlar:

- Kolonizasyonun enfeksiyondan ayrılması
- Dış kaynaklı nükleik asit
- Yöntem standardizasyonu
- Verilerin saklanması, korunması, analizi ve değerlendirilmesi



Simner *et al*. (2018) Understanding the Promises and Hurdles of Metagenomic Next-Generation Sequencing as a Diagnostic Tool for Infectious Diseases . *Clin. Infect. Dis.*

Journal of the Pediatric Infectious Diseases Society Advance Access published January 6, 2017

Journal of the Pediatric Infectious Diseases Society

BRIEF REPORT

Neurobrucellosis: Unexpected Answer From Metagenomic Next-Generation Sequencing

Kanokporn Mongkolrattanothai[1,a], Samia N. Naccache[2,3,5,a], Jeffrey M. Bender[1], Erik Samayoa[3,5], Elizabeth Pham[3,5], Guixia Yu[3,5], Jennifer Dien Bard[2], Steve Miller[3,5], Grace Aldrovandi[1,6], and Charles Y. Chiu[3-5]

Table 1. Patient's Microbiological Workup

Variable	Mexico	Los Angeles (Children's Hospital Los Angeles)		
		First Admission (February 26, 2015)	Hospital Day 8 (March 6, 2015)	Second Admission (March 9, 2016)
Cerebrospinal fluid				
Color	Colorless	Colorless	Colorless	Colorless
Turbidity	Slight	Clear	Hazy	Clear
Red cell count (per mm^3)	-	0	1	0
White cell count (per mm^3)[a]	183	137	176	0
Differential count (%)				
Neutrophils	0	5	53	0
Lymphocytes	0	91	38	0
Monocytes	100	4	9	0
Protein (mg/dL)[b]	270	200	131	23
Glucose (mg/dL)[c]	25	25	25	45
Blood glucose (mg/dL)[d]	84	86	-	87
Microbiological tests				
Staining[e]	Rare Gram-positive cocci	No organisms	No organisms	No organisms
Culture[f]	No growth	No growth	No growth	No growth
Cytomegalovirus DNA (PCR)	Negative	-	-	-
Enterovirus DNA (PCR)	Negative	Negative	-	-
Epstein-Barr virus DNA (PCR)	Detected	Negative	Detected	-
Herpes simplex virus (PCR)	Negative	Negative	-	-
Human herpesvirus 6 (PCR)	Negative	-	Negative	-
Human herpesvirus 7 (PCR)[g]	Detected	-	Detected	-
Mycobacterial DNA (MTB-PCR)[h]	-	Negative	Negative	-
VDRL	Nonreactive	-	-	-
mNGS testing (UCSF Clinical Microbiology Laboratory)	-	-	*Brucella* detected	-
Blood				
Brucella ELISA IgM	Negative	-	-	-
Brucella EIA IgM[i]	-	Negative	-	Negative
Brucella EIA IgG[i] [<0.80, negative; 0.8–1.09, equivocal; ≥1.10, positive]	-	Positive (4)	-	Positive (4.68)
Brucella agglutinins[j] [≥1:80, positive]	-	-	-	Positive (1:80)
Coccidioidal antibody[k]	-	Negative	-	-
Cryptococcal antigen	-	Negative	-	-
HIV antibody and p24 antigen	-	Negative	-	-
Interferon gamma release assay (QuantiFERON-TB Gold)	-	Negative	-	Negative
RPR	Negative	Negative	-	-
Urine				
Histoplasma antigen	-	Negative	-	-

MALDI-TOF MS

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

- Rutin laboratuvarlara 2009 yılında girdi
- Bugün >2.000 laboratuvarda kullanılıyor

- Bakteriler, mayalar, mikobakteriler, filamentöz mantarlar

- >2.500 tür

- Avantajlar: masraflarda azalma, pozitif kan kültüründen doğrudan tanımlama, tiplendirme, antibiyotik duyarlılık testi (MBT ASTRA)

Kostrzewa M. (2018) Application of the MALDI Biotyper to clinical microbiology: progress and potential. Expert Review of Proteomics.

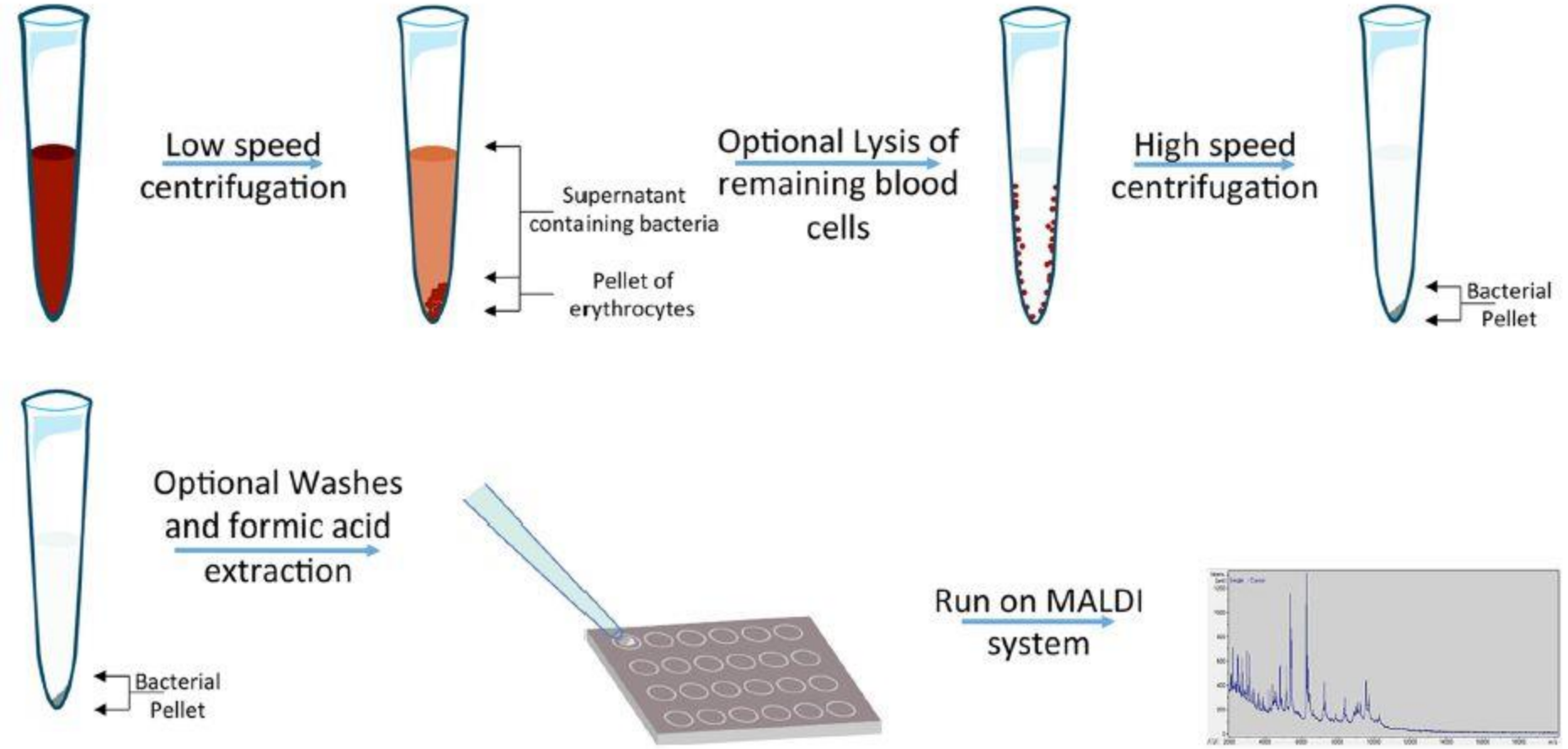


FIG 1 Procedures between studies to perform identification directly from positive blood cultures using MALDI-TOF MS can differ greatly but usually follow a similar workflow. A sample of blood culture is drawn from the bottle and is centrifuged to pellet the erythrocytes. In some studies, after the supernatant is collected, an optional lysis of remaining blood culture cells is performed to further clean the sample to produce less background. High-speed centrifugation is performed to pellet the remaining bacteria. This pellet can be washed and centrifuged a few times to improve the purification of bacterial pellets. Once purified, either an extraction can be performed or the bacteria can be added to a target plate and tested on a MALDI system for identification.

Faron *et al.* (2017) Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Use with Positive Blood Cultures: Methodology, Performance, and Optimization. *J. Clin. Microbiol.*

TABLE 1 Summary of MALDI-TOF MS blood culturing methods

Source (reference)	MALDI used	Starting blood vol (ml)	Removal blood cell spin	Lysis	Centrifugation step	Waste	Wash step(s)	Centrifugation step	Pellet loading	Hands-on time	Overall concordance (%)
Chen et al. (21) and Martiny et al. (22)	Vitek MS and microflex LT Biotyper	1	None	Blood added to 200 μl of 5% saponin lysis; vortex and wait 5 min	16,600 × *g*, 1 min	Supernatant	1 ml of deionized H_2O	16,600 × *g*, 1 min	Dry and smear to target plate	20 min	Genus, 86.4–96.7; species, 73.7–80.4
Prod'hom et al. (18)	microflex LT Biotyper	5 into 40 H_2O	1,000 × *g*, 10 min	Resuspend pellet in 1 ml of 0.15 M NH_4Cl, 1 mM $KHCO_3$	140 × *g*, 10 min	Supernatant	2 ml of H_2O	140 × *g*, 10 min	Dry and smear to target plate	~40 min	99 concordant when score >1.7 obtained; 78.7 obtained score
Christner et al. (23)	microflex LT Biotyper	6	140 × *g*, 10 min	4 ml of supernatant mixed with 2 ml of H_2O	1,000 × *g*, 5 min	Supernatant	1 ml of distilled H_2O, resuspend 300 μl of distilled H_2O plus 900 μl of ethanol	13,000 × *g*, 2 min	Dry resuspended in 50 μl of 70% formic acid and 50 μl of acetonitrile (centrifuged); 1 μl added to target plate	~20 min	Species, 87
Ferreira et al. (19)	Autoflex III	4	2,000 × *g*, 30 s	None	15,500 × *g*, 5 min	Supernatant	1 ml of distilled H_2O, resuspend 300 μl of distilled H_2O plus 900 μl of ethanol	15,500 × *g*, 5 min	Dry resuspended in 50 μl of 70% formic acid and 50 μl acetonitrile (centrifuged); 1 μl added to target plate	<30 min	GN, 96.6 genus, 83.3 species; GP, 64.8 genus, 31.8 species
Monteiro et al. (20)	Vitek MS	5	500 rpm, 20 min	1 ml of supernatant was centrifuged at 13,000 rpm for 1 min and resuspended in 200 μl of 0.1% TFA	13,000 rpm, 1 min	Supernatant	None	NA[a]	Dry resuspended in 50 μl of 70% formic acid and 50 μl acetonitrile, wait 20 min (centrifuged); 1 μl added to target plate	<50 min	GN, 99 species; GP, 86.3 genus, 82.3 species
Bazzi et al. (16)	Vitek MS	1.5	None	50 μl of 10× Triton	13,000 rpm, 1 min at 4°C	Supernatant	1.5 ml of 0.9% saline	13,000 rpm, 1 min at 4°C	Various extractions	~20 min	GN, 95–100 genus, 90.9 species; GP, 76.7–90 genus, 76.4–89 species
Moussaoui et al. (17)	Biflex III	1.5	Serum separator tube 500 × *g*, 10 min	1.5 ml of sterile H_2O	300 × *g*, 1 min	Pellet	1 μl of supernatant transferred to a microtube	10,000 × *g*, 2 min	Ethanol-formic acid extraction	24 samples/80 min	GN, 91.1 species; GP, 89.0 species
La Scola and Raoult (32)	Autoflex III	1	(i) 500 rpm, 15 min; (ii) 500 rpm, 5 min	None	(i and ii) 14,000 rpm, 20 min	Supernatant	(i and ii) 1 ml of distilled H_2O	(i and ii) 14,000 rpm, 20 min	(i) 5 μl of acetonitrile plus 5 μl for 15 min (centrifuge), 2 μl added to target plate; (ii) 5 μl formic acid for 5 min, add 5 μl acetonitrile (centrifuge); 2 μl added to target plate	<2 h	(i) GN, 94 species; GP, 37 species; (ii) GN, 87 species; GP, 67 species
BD Sepsityper (28, 55, 56)	microflex LT Biotyper and microflex II	1	None	200 μl of lysis buffer	13,000 rpm, 2 min	Supernatant	1 ml of washing buffer	13,000 rpm, 1 min	Unspecified	~20 min	GN, 87.7–97.8 species; GP, 76–85.7 species; yeast, 66 species
bioMérieux filter system (24)	Vitek MS	2	None	1 ml of lysis buffer	Add to vacuum filter	Supernatant	3 × 1 ml of washing buffer	Vacuum liquid	Smear to target plate	<15 min	GN, 84 species; GP, 74.5 species; yeast, 94.1 species

[a]NA, not applicable.

Hızlı Antibiyotik Duyarlılık Testleri

≤ 8 saat

Accelerate PhenoTest™ BC

Örnek: pozitif kan kültürü şişesi
Yöntem: zaman atlamalı mikroskopi

ADT sonuçları 7 saat + kan kültürü inkübasyon süresi

Avantajlar:
FDA onaylı
Çok sayıda literatür desteği
Altın standart (sıvı mikrodilüsyon) ile iyi kategori uyumu

Dezavantajlar:
Tek örnek/cihaz
Masraf
Zor suşlar için performansı bilinmiyor?
Kolistin

Pantel et al. JAC 2018
Charnot-Katsikas et al. JCM 2018
Pancholi et al. JCM 2018

Hızlı Antibiyotik Duyarlılık Testleri – CLSI'nin yaklaşımı

- Pozitif kan kültürü şişesindeki sıvı besiyerinin disk difüzyon testi için inokulum olarak kullanıldığı 'direkt disk difüzyon' testi

- Seçilmiş 20 köken içeren set (*Enterobacteriaceae*, *A. baumannii*, *P. aeruginosa*)

- Üç farklı kan kültürü sistemi değerlendirilmiş:

  BacT/ALERT, BACTEC ve VersaTREK

Chandrasekaran *et al.* (2017) Direct from-blood culture disk diffusion to determine antimicrobial susceptibility of Gram negative bacteria: Preliminary report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. *J. Clin. Microbiol.*

Hızlı Antibiyotik Duyarlılık Testleri – CLSI'nin yaklaşımı

	Kategori uyumu	VME (R → S)	ME (S → R)
BacT/ALERT	%87.8	-	%3.0
BACTEC	%88.4	-	%2.3
VersaTREK	%92.2	-	%1.7

Disk difüzyon sonuçları 6. saaat ve 16-18. saatte okunmuş.

Kökenlerin %19.9'u 6. saatte değerlendirilememiş (zayıf üreme)

Chandrasekaran *et al.* (2017) Direct from-blood culture disk diffusion to determine antimicrobial susceptibility of Gram negative bacteria: Preliminary report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. *J. Clin. Microbiol.*

Hızlı Antibiyotik Duyarlılık Testleri – EUCAST'ın yaklaşımı

Doğrudan pozitif kan kültürü şişelerinden disk difüzyon yöntemi ile hızlı ADT

EUCAST standart yöntemi.
Uygulama tarihi 2018

EUCAST RAST kan kültürü saha çalışması

44 laboratuvar katılacağını belirtti

40 laboratuvar katıldı ve tüm türler için sonuç gönderdi

Danimarka (3), Finlandiya (3), İzlanda (1), İrlanda (1),

Norveç (11) ve İsveç (21)

Kan kültürü sistemleri:

BD BACTEC n=17
bioMerieux BacT/ALERT n=23

Disk üreticisi: 4

MH üreticisi: 6

Referans: Sıvı mikrodilüsyon + standart EUCAST disk difüzyon (16-20 saat)

Species	Number
E. coli	436
K. pneumoniae	64
Ps. aeruginosa	37
Other gram negatives	52
S. aureus	270
Coagulasnegative staph.	357
S. pneumoniae	35
Total number	**1251**

RAST vs. standart disk difüzyon - *E. coli*

	E. coli **(n=386)** Cefotaxime, ceftazidime, piperacillin-tazobactam, meropenem, ciprofloxacin,			***E. coli*** **(n=386)** Piperacillin-tazobactam excluded		
Incubation time	**4h**	**6h**	**8h**	**4h**	**6h**	**8h**
Number of possible tests[a]	3 088	3 088	3 088	2 702	2 702	2 702
Number of performed tests[b]	3 034	3 027	2 768	2 651	2 645	2 419
Number of zones registered[c]	2 756	2 993	2 752	2 415	2 613	2 404
	Categorical agreement (%)					
Correct	77	81	84	88	93	95
mE	0.3	0.1	0.1	0.2	0.2	0.1
ME	1.6	0.4	0.2	1.8	0.5	0.3
VME	0.1	0.1	0.1	0.1	0.1	0.1
ATU	20	18	16	10	6.2	4.0

Yayınlanmamış veri. Jonasson E, Matuschek E, Kahlmeter G.

RAST vs. standart disk difüzyon - *S. aureus*

	***S. aureus* (n=242)** Cefoxitin, norfloxacin, erythromycin, gentamicin		
Incubation time	**4h**	**6h**	**8h**
Number of possible tests[a]	968	968	968
Number of performed tests[b]	952	956	892
Number of zones registered[c]	623	880	844
	Categorical agreement (%)		
Correct	66	92	95
mE	0.0	0.0	0.0
ME	8.5	0.3	0.4
VME	0.2	0.3	0.5
ATU	25	7.2	4.0

Yayınlanmamış veri. Jonasson E, Matuschek E, Kahlmeter G.

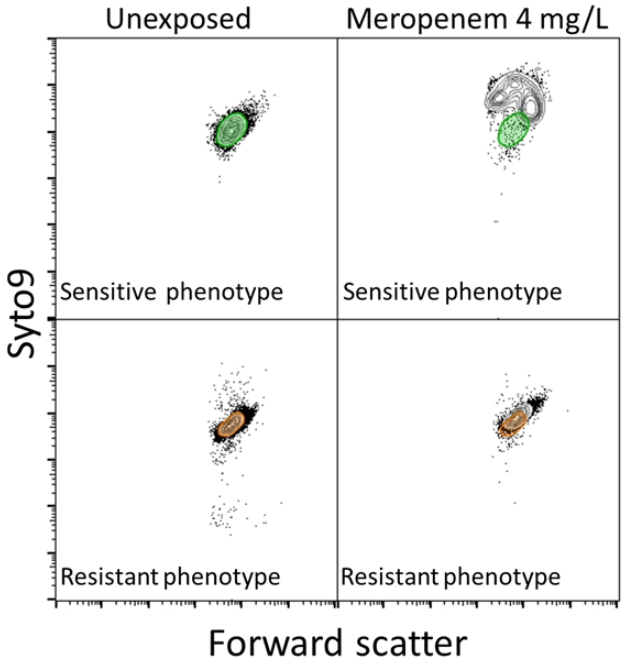
< 4 saat

Akım sitometresi ile ADT - FAST

Meropenem, *K. pneumoniae*
Uygulama süresi = 30 dak

MİK belirlenmesi
Morfolojik değişikliklere dayanıyor

Toplam sonuç süresi
= 90-150 dakika




SCIENTIFIC REPORTS

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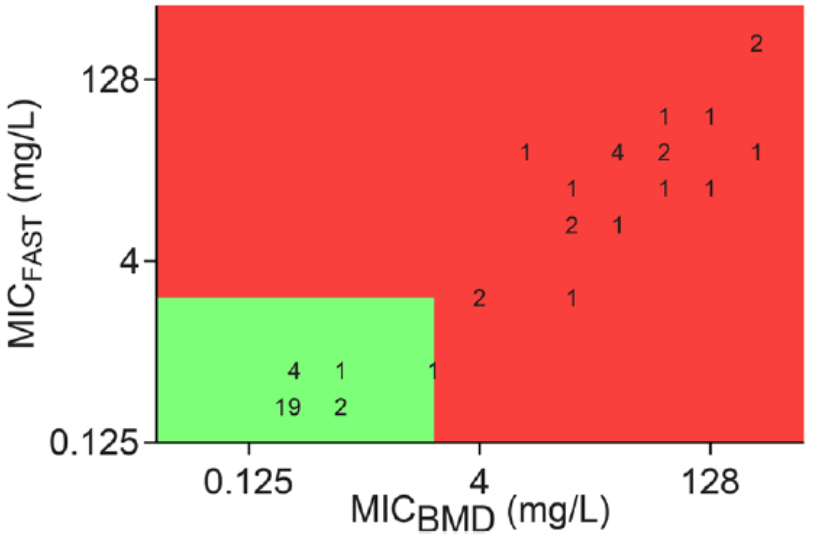
Rapid susceptibility profiling of carbapenem-resistant *Klebsiella pneumoniae*



Mulroney et al. Sci Rep 2017.

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

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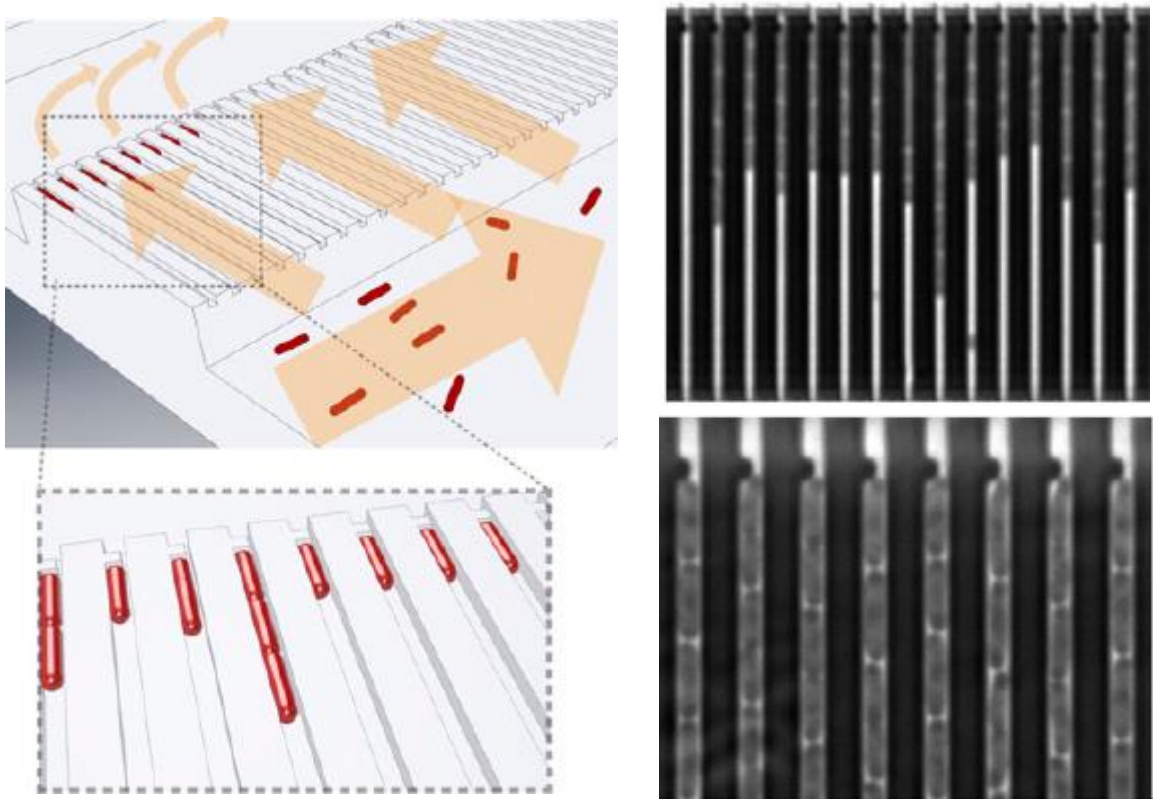

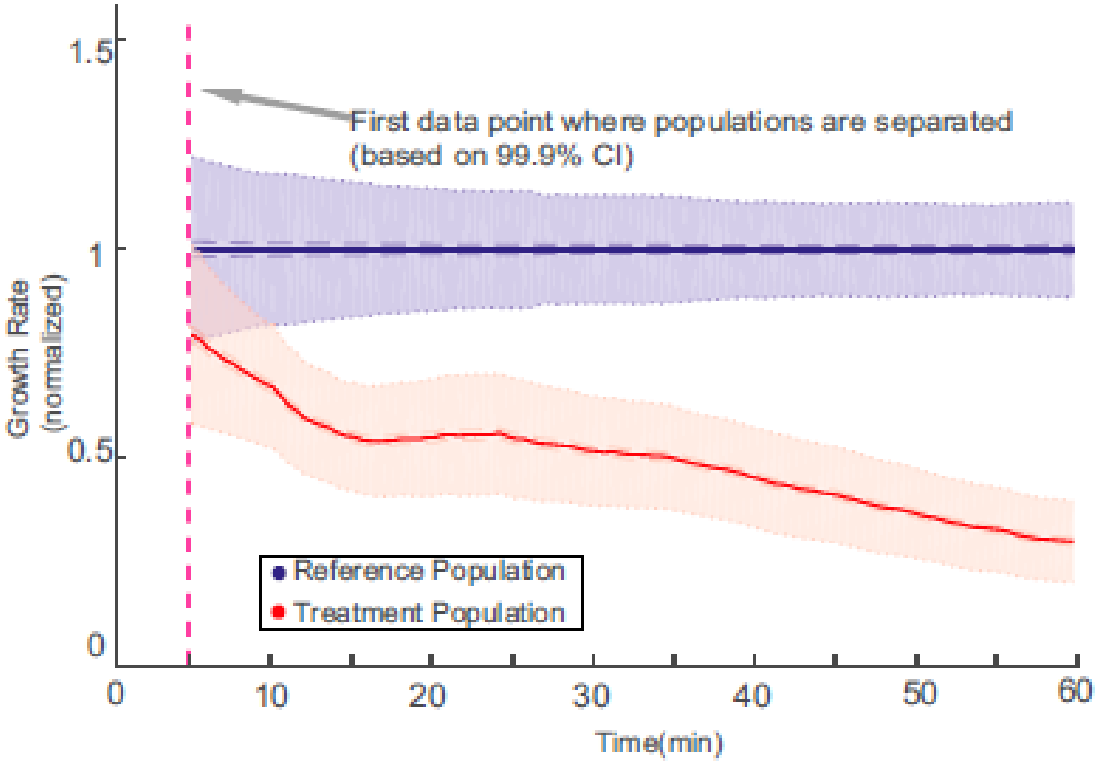

Baltekin et al. PNAS Aug 22, 2017.

Özetle

- Gelişen biyoteknolojik yöntemler ile enfeksiyon hastalıklarının tanısı için ihtiyaç duyulan

  - hız
  - maliyet-etkinlik
  - duyarlılık ve özgüllük
  - kolay ulaşılabilirlik (hasta başı testler)

  Özelliklerini karşılayan mikrobiyoloji testleri yakın gelecekte yaygın kullanıma girecek.

Hızlı ADT konusunda yayınlanmamış çalışmalarına ait sonuçları paylaşmama izin verdikleri için
Gunnar Kahlmeter ve Oskar Ekelund’a teşekkürlerimle.