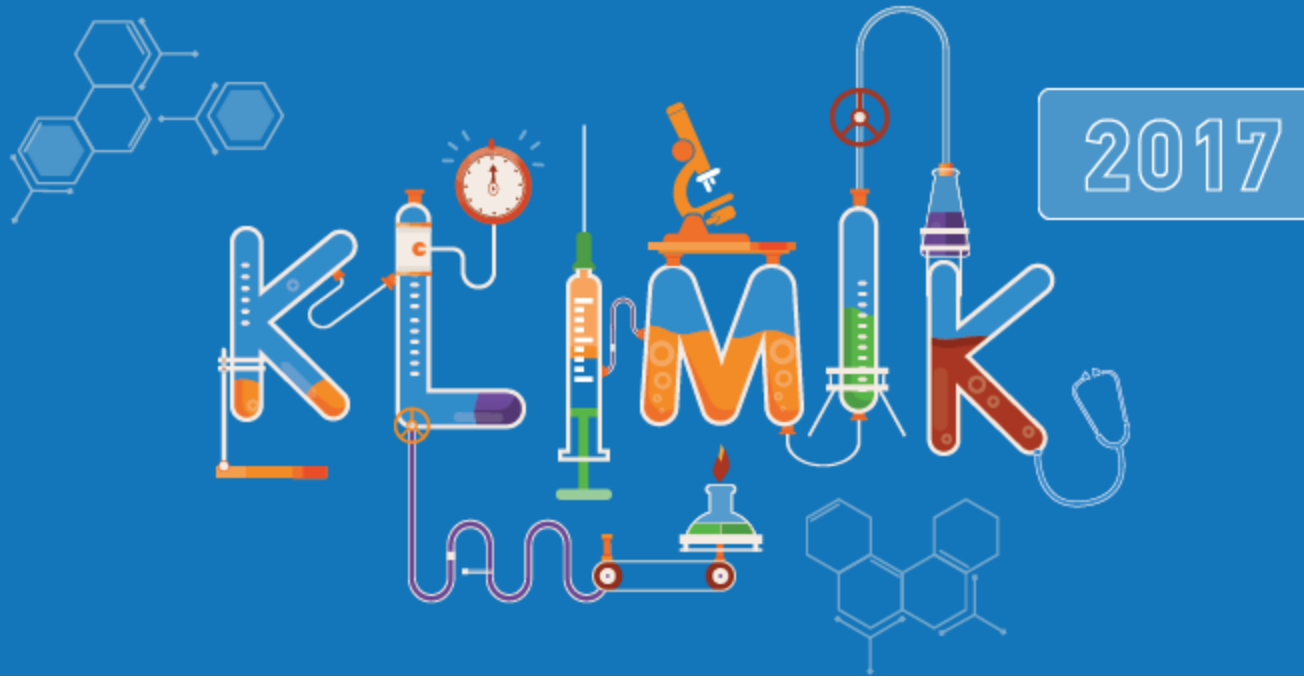




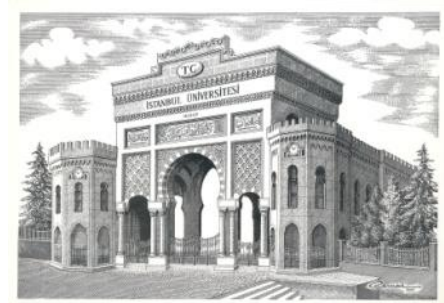
Mikrobiyolojide Güncel Testler

Moleküler Testlerde Tanı Yönetimi

Mert A. Kuşkucu PhD

İ.Ü. Cerrahpaşa Tıp Fakültesi

Tıbbi Mikrobiyoloji Anabilim Dalı



Sunum Planı;

Moleküler
Testler
Gelişen
Teknoloji



Tanıda
Değişen
Yaklaşımlar



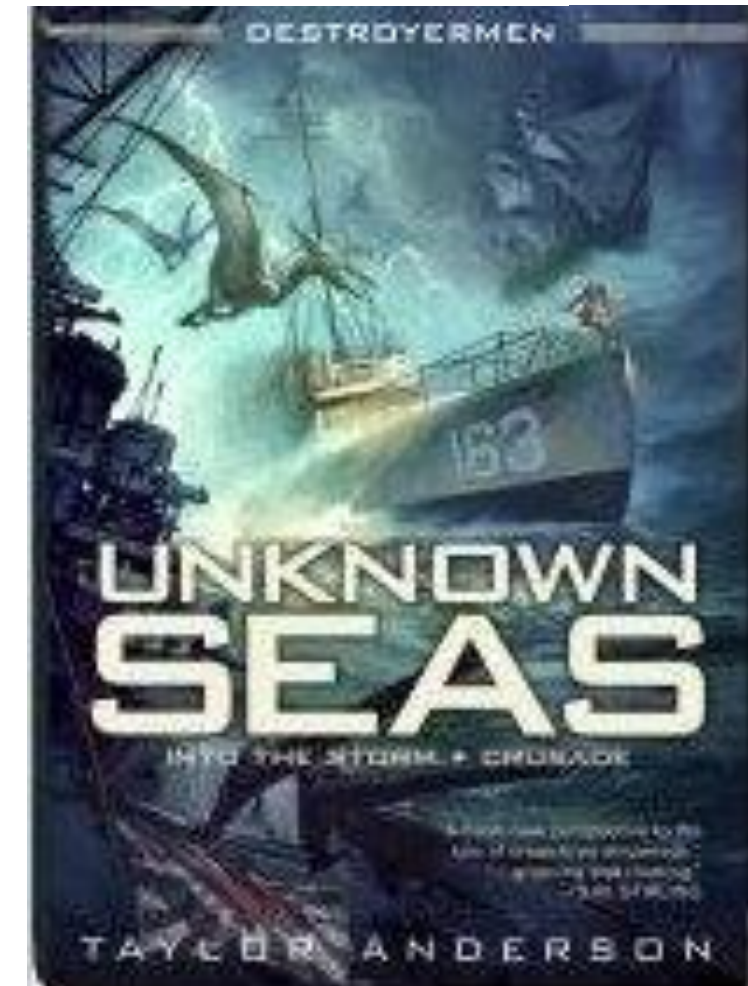
Moleküler
Testlerde Tanı
Yönetimi

GUEST COMMENTARY

Clinical Microbiology in the Year 2025

W. Michael Dunne, Jr.,^{1*} J. Keith Pinckard,¹ and Lora V. Hooper²

*Departments of Pathology and Immunology¹ and Molecular Biology and Pharmacology,²
Washington University School of Medicine, St. Louis, Missouri 63110*



The clinical microbiology laboratory is in the midst of a diagnostic revolution.
Messacar K. J. Clin. Microbiol. doi:10.1128/JCM.02264-16



Moleküler Testler = Vazgeçilmez Alet Kutusu

- Nükleik Asit Tabanlı Testler;
 - Nükleik Asit Eldesi + Amplifikasyon
 - Minyatürizasyon; “Stand-Alone” sistemler; “Point of Care” Teknolojileri
- DNA Dizi Analiz Yöntemleri
- Kütle Spektrofotometresi
- “Phage Display”
- Değişen Tanı Yaklaşımı

Amplifikasyon Yöntemleri

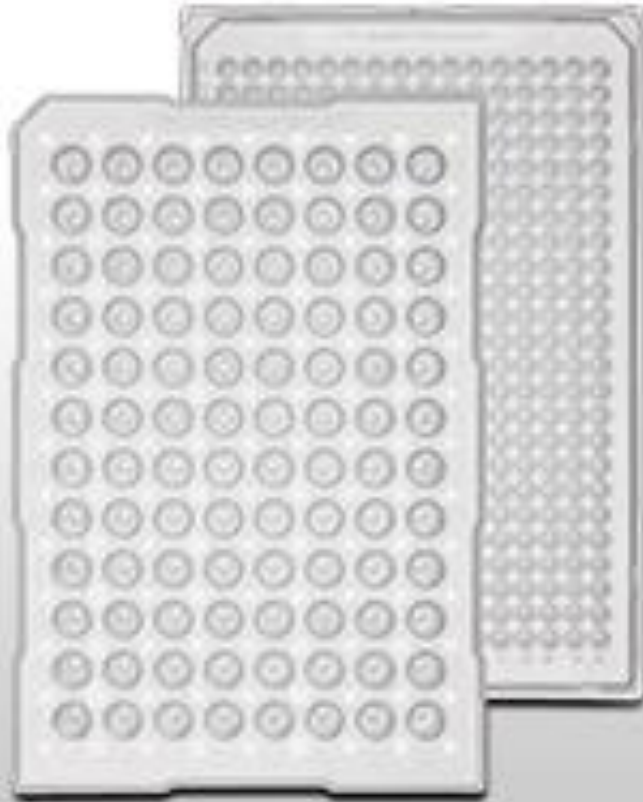
Döngüsel Isı Profili Kullananlar

PCR
Real Time PCR

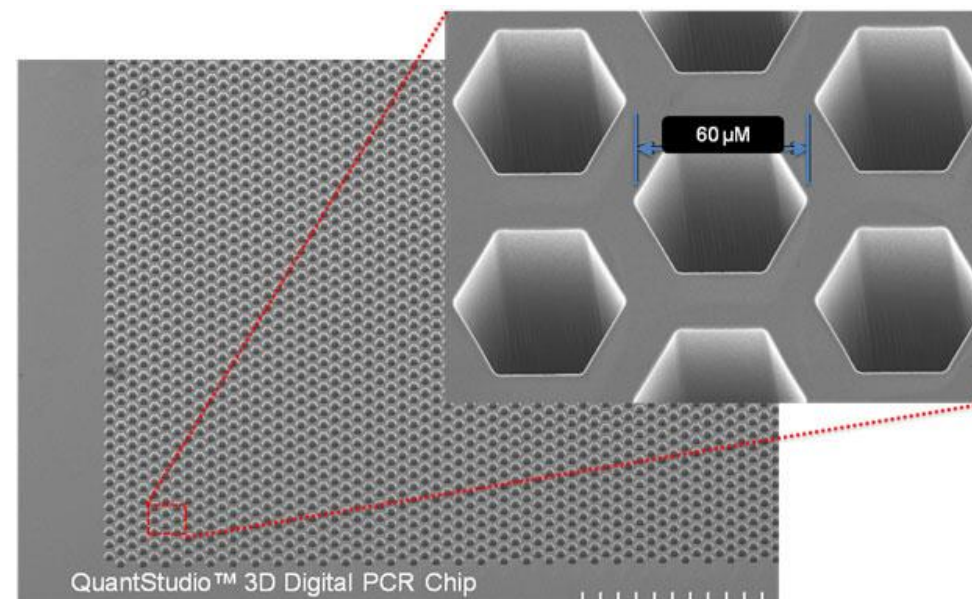
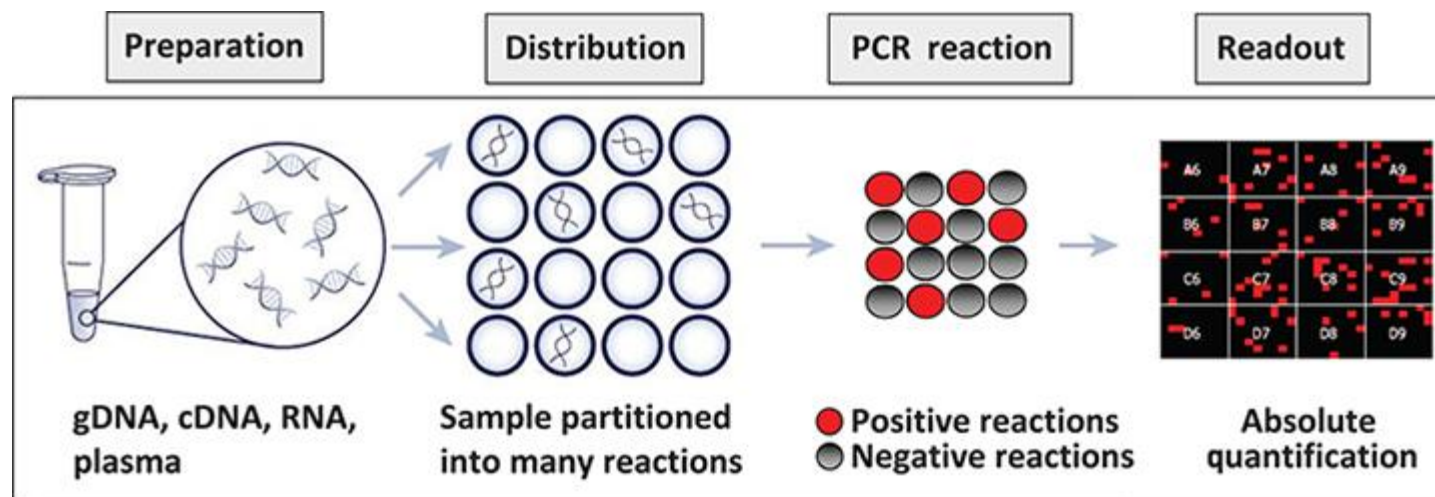
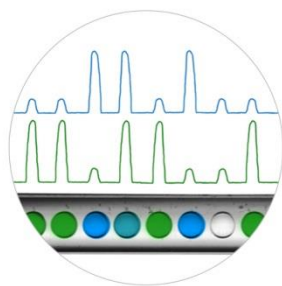
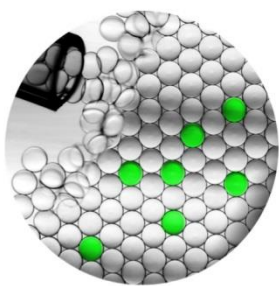
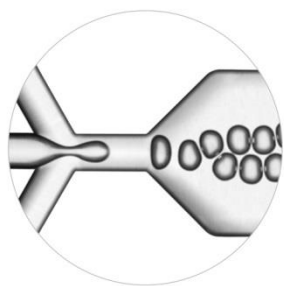
İzotermal Yöntemler

LAMP
RPA
RCA
Helikaz Depended
Amplifikasyon
LCR
.
.
.

Real Time PCR; Çoklu PCR; Digital PCR



Digital PCR



Assays	Samples
12	384
24	216
36	144
48	108
54	96
72	72
80	64
96	54
120	42
144	36
216	24
248	20
296	16
384	12

Many Flexible Configurations

With many different preprogrammed dispense configurations on the MSND printing your assays in your lab has never been easier. Print in your lab in about 30 minutes and begin your experiments today, no need to wait.

A new ordering interface!

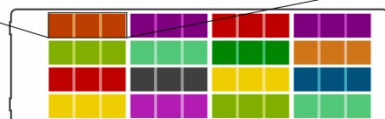
Design your own plate, building from inventoried, pre-designed, and custom TaqMan® Gene expression assays. Minimum order quantity is dependent upon OpenArray® format.

Select your array format:

Format	Number of Assays	Number of Samples	Minimum Order Qty		
18	18	48	1 (10 pack)	View Layout	Select
56	56	48	1 (10 pack)	View Layout	Select
112	112	24	2 (10 pack)	View Layout	Select
168	168	16	3 (10 pack)	View Layout	Select
224	224	12	4 (10 pack)	View Layout	Select

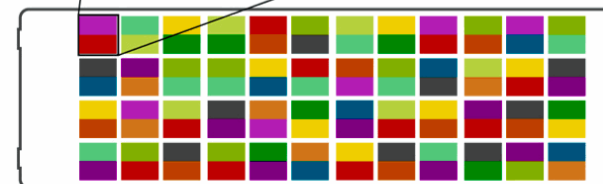
FOR GT CUSTOM 192

	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8
a	1	2	3	4	5	6	7	8	a	9	10	11	12	13	14	15	16	a	17	18	19	20	21	22	23	24
b	25	26	27	28	29	30	31	32	b	33	34	35	36	37	38	39	40	b	41	42	43	44	45	46	47	48
c	49	50	51	52	53	54	55	56	c	57	58	59	60	61	62	63	64	c	65	66	67	68	69	70	71	72
d	73	74	75	76	77	78	79	80	d	81	82	83	84	85	86	87	88	d	89	90	91	92	93	94	95	96
e	97	98	99	100	101	102	103	104	e	105	106	107	108	109	110	111	112	e	113	114	115	116	117	118	119	120
f	121	122	123	124	125	126	127	128	f	129	130	131	132	133	134	135	136	f	137	138	139	140	141	142	143	144
g	145	146	147	148	149	150	151	152	g	153	154	155	156	157	158	159	160	g	161	162	163	164	165	166	167	168
h	169	170	171	172	173	174	175	176	h	177	178	179	180	181	182	183	184	h	185	186	187	188	189	190	191	192



FOR GT CUSTOM 32

	1	2	3	4	5	6	7	8
a	1	2	3	4	5	6	7	8
b	9	10	11	12	13	14	15	16
c	17	18	19	20	21	22	23	24
d	25	26	27	28	29	30	31	32
e	1	2	3	4	5	6	7	8
f	9	10	11	12	13	14	15	16
g	17	18	19	20	21	22	23	24
h	25	26	27	28	29	30	31	32



Journal content

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[Focuses and Supplements](#)

[Methagora blog](#)

TECHNOLOGY FEATURE

Nature Methods **7**, 351 - 356 (2010)
doi:10.1038/nmeth0510-351

Clever PCR: more genotyping, smaller volumes

Monya Baker¹

With microfluidics and multiplexing, researchers can get more information from PCR products in less time and with fewer reagents.

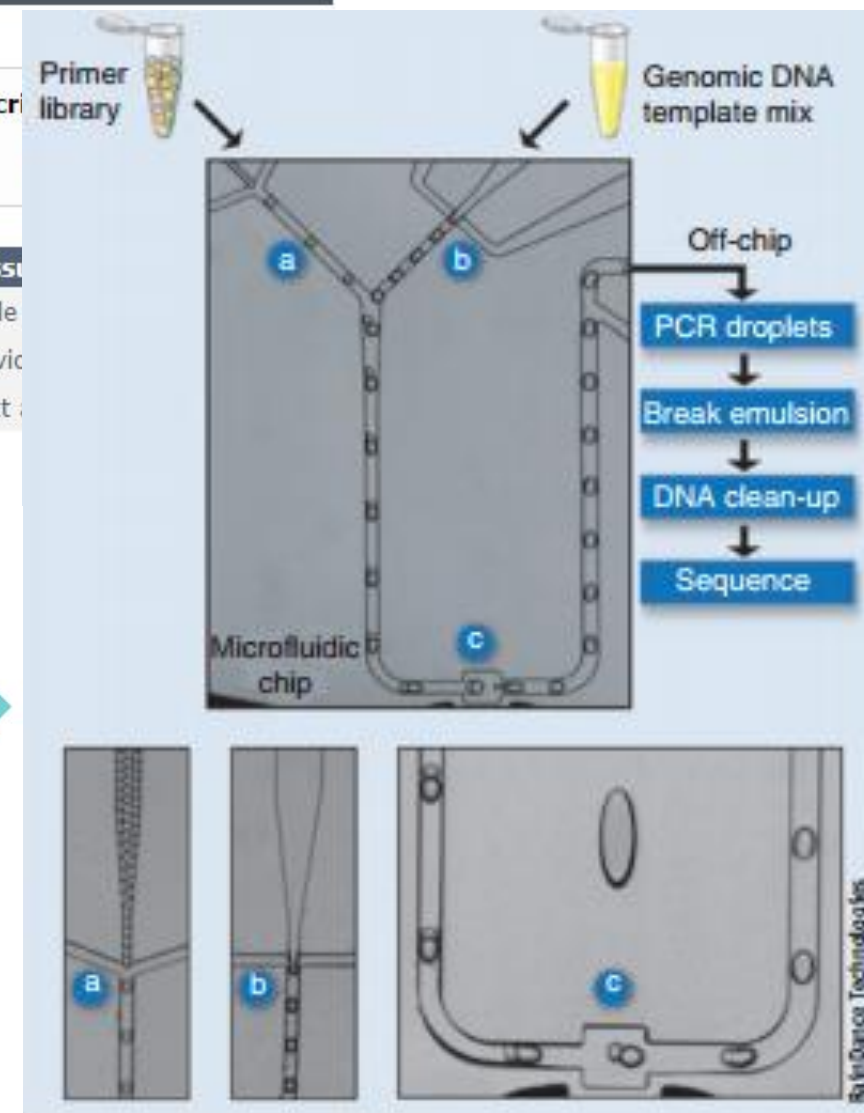
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RainDance creates tiny droplets as vessels for PCR amplification.

Fixed or custom primer panels

Dispense samples and indexed primers

Perform singleplex PCR

Pool indexed samples

Purify and Sequence

1

2

3

4

5



SmartChip is configured for multiple samples and contain target specific primers



Multi-Sample Nanodispenser dispenses samples & indices onto chip



96 well Thermal Cycler with SmartChip PCR adapter



Amplicons extraction using proprietary method*



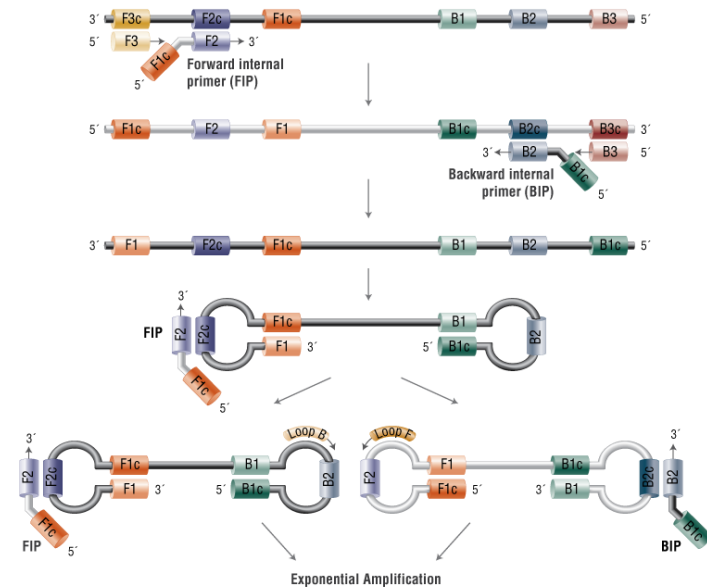
Indexes sequencing ready amplicons are purified & NGS ready

İzotermal Yöntemler

- LAMP
- TMA, NASBA
- RPA
- RCA
- Helikaz Depended Amplifikasyon

- LCR

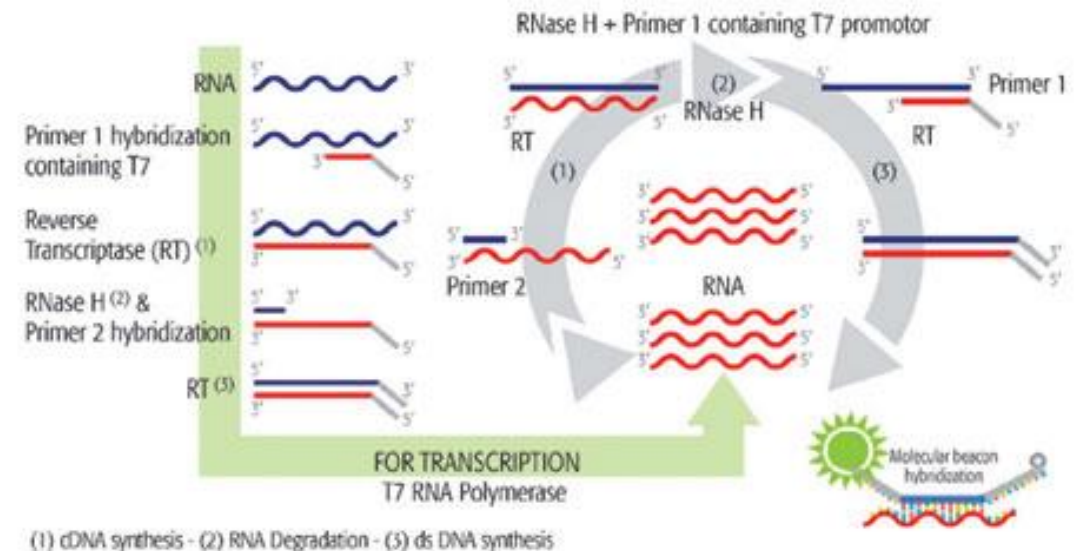
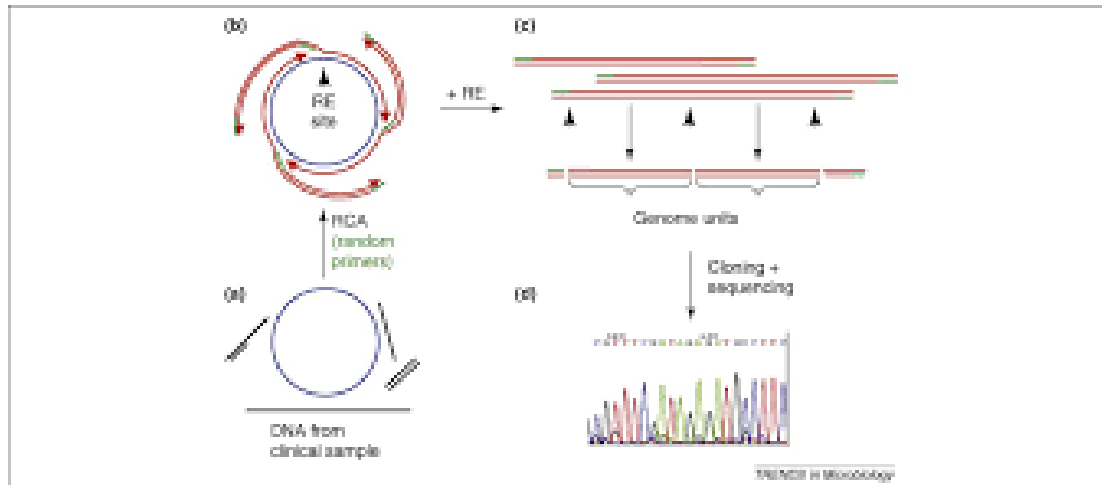
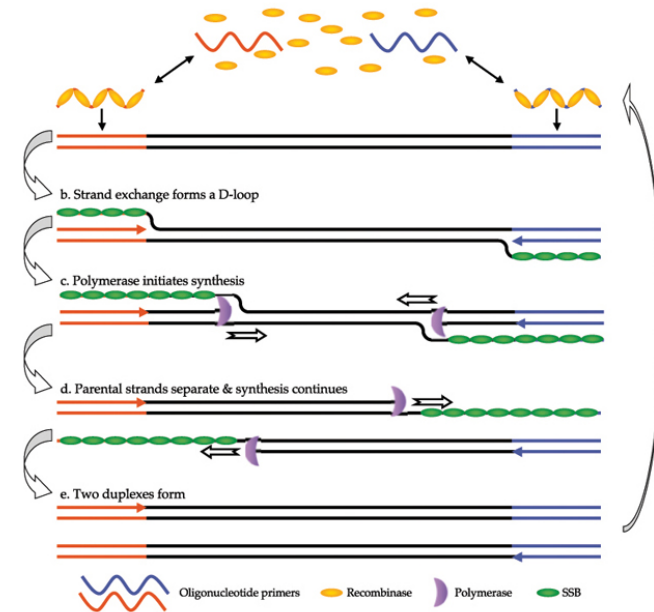
- .
- .
- .



The RPA Cycle

All steps operate at low constant temperature (optimum 37°C)

a. Recombinase / oligonucleotide primer complexes form and target homologous DNA



Amplifikasyon Yöntemleri ve Sensör Teknolojisi

Döngüsel Isı Profili Kullananlar

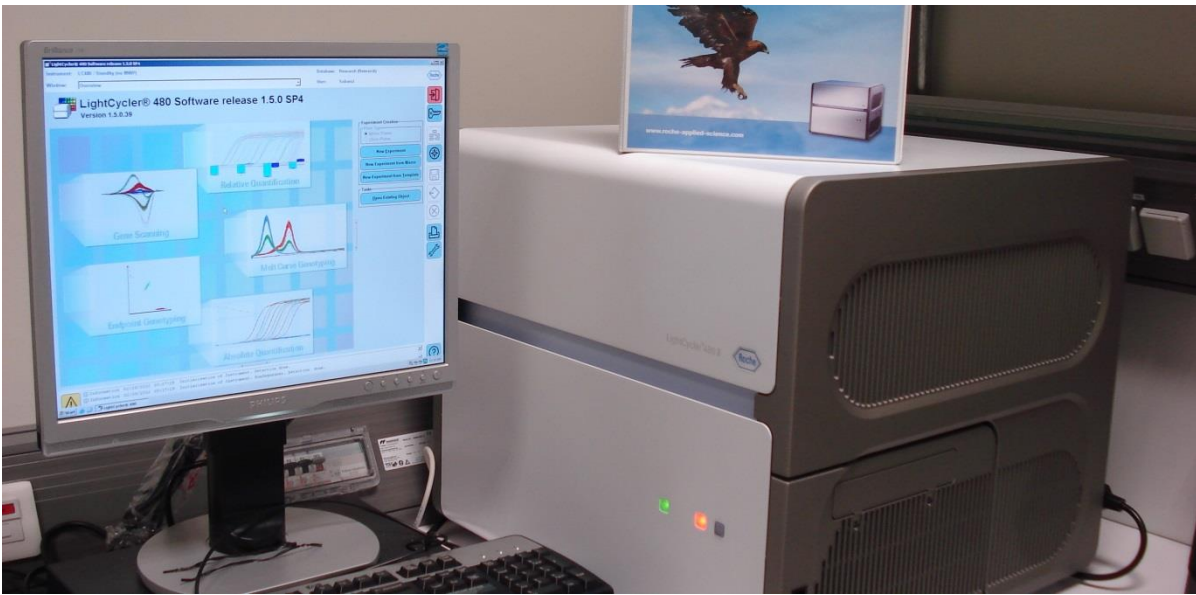
PCR
Real Time PCR

İzotermal Yöntemler

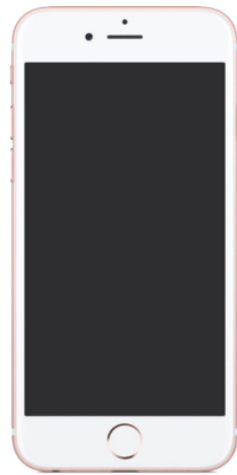
LAMP
RPA
RCA
Helikaz Depended
Amplifikasyon
LCR
.
.
.

Saptama, Sensörler

Fluorescence based real-time
detection
Electrophoresis
Lateral Flow
DNA hybridization microarray
Electrochemical method



Monochrome CCD camera
1024 x 1344 pixels = **1,3 Megapiksel**



12 Megapiksel



16 Megapiksel

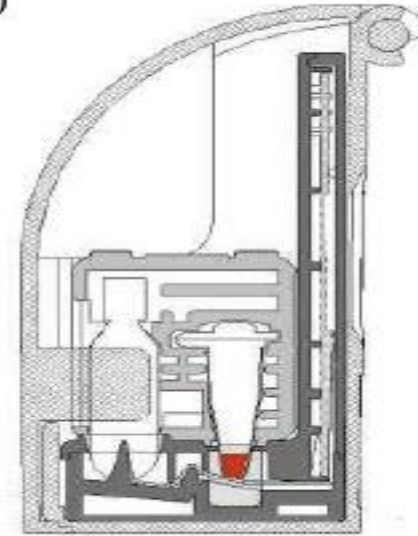


13 Megapiksel

“Stand-Alone” Sistemler ; XCP nucleic acid detection device (Ustar Biotechnologies)/BESt Cassette (BioHelix)

- Amplifikasyon ürününü lateral flow stribi aktarıyo.
- Ürünler biotin-FAM, biotin-DIG işaretli olabiliyo.
- Amplifikasyon tüpü doğrudan kartuşa yerleştiriliyor
- IsoAmp HSV assay FDA onaylı

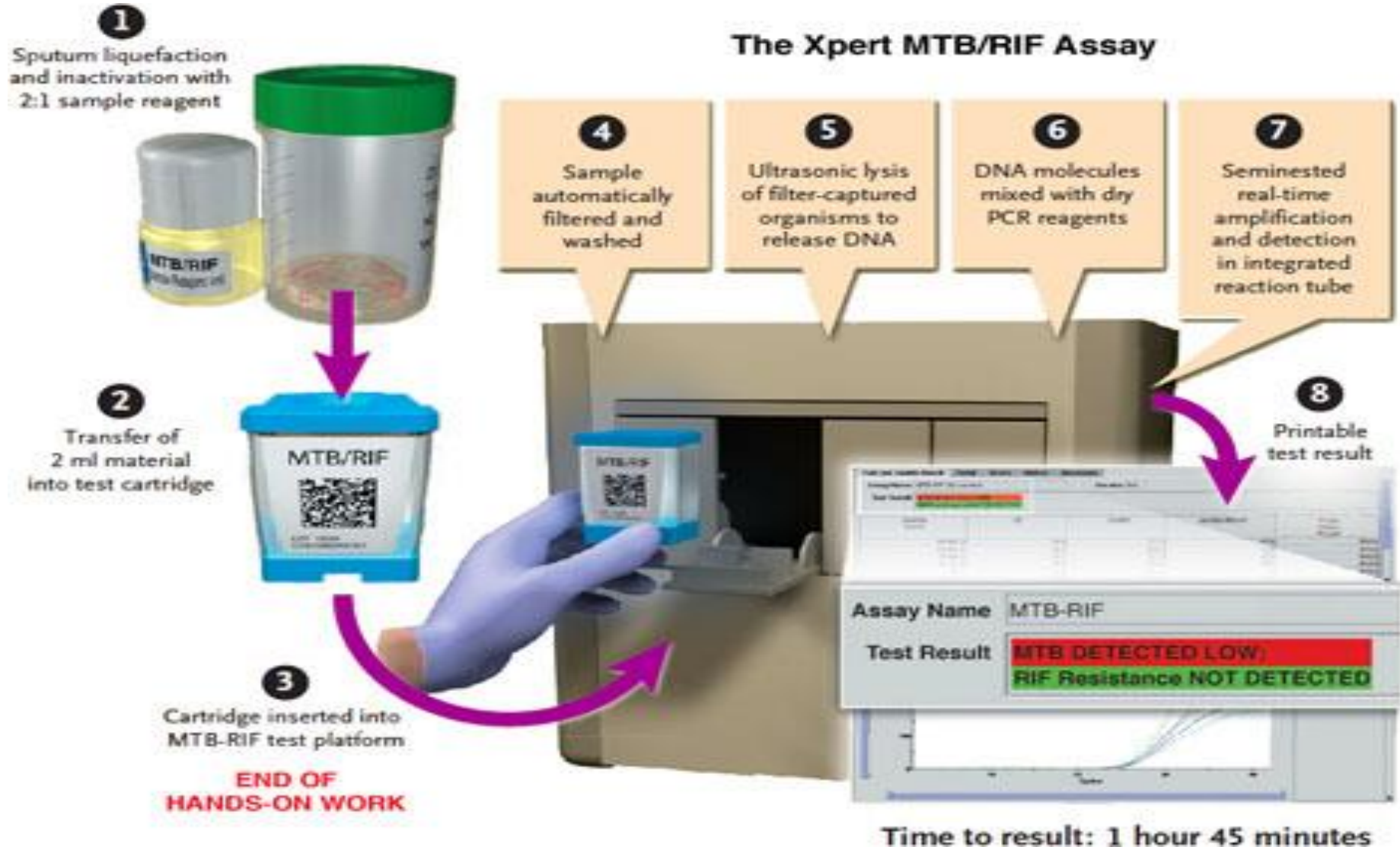
(A)



(B)

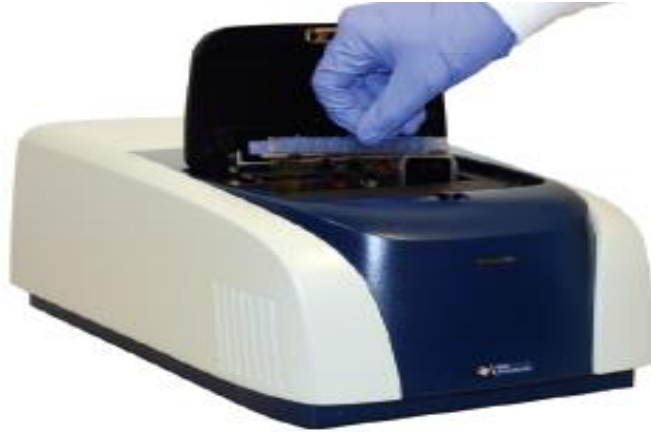


“Stand-Alone” Bütünleşik Sistemler ; GeneXpert (Cepheid)



“Stand-Alone” Bütünleşik Sistemler ; FilmArray (Biofire)

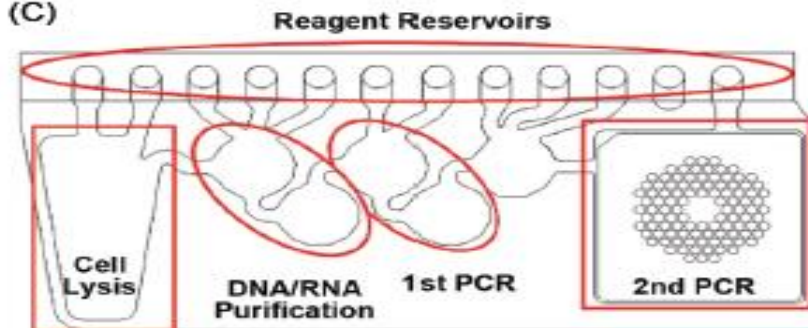
(A)



(B)

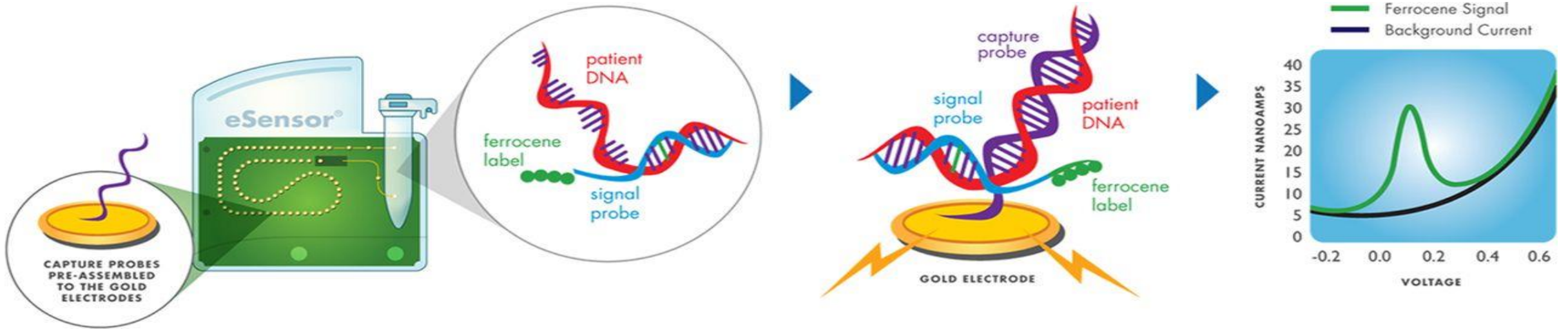


(C)



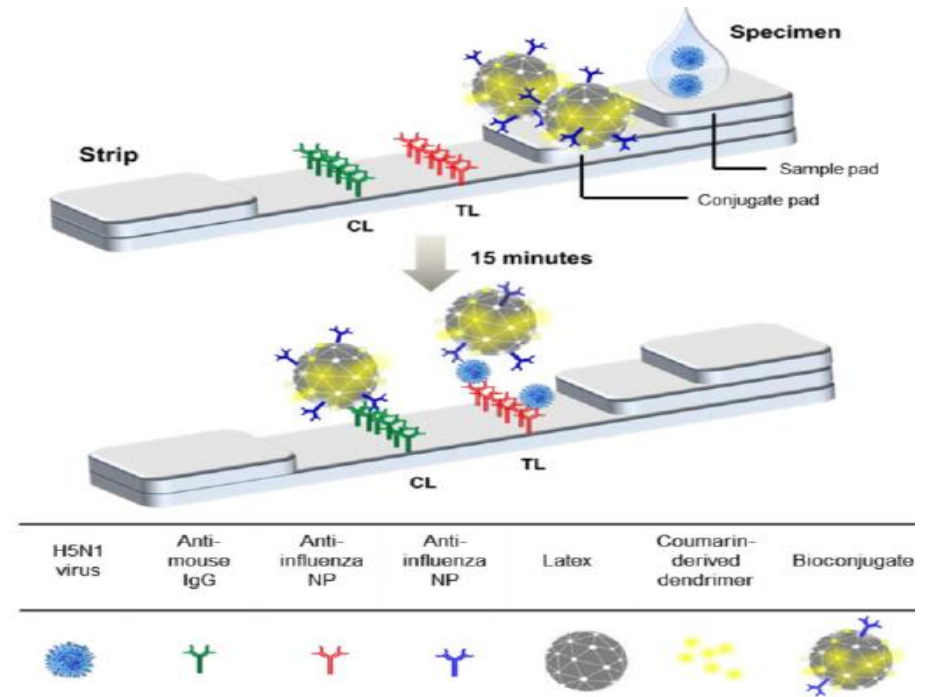
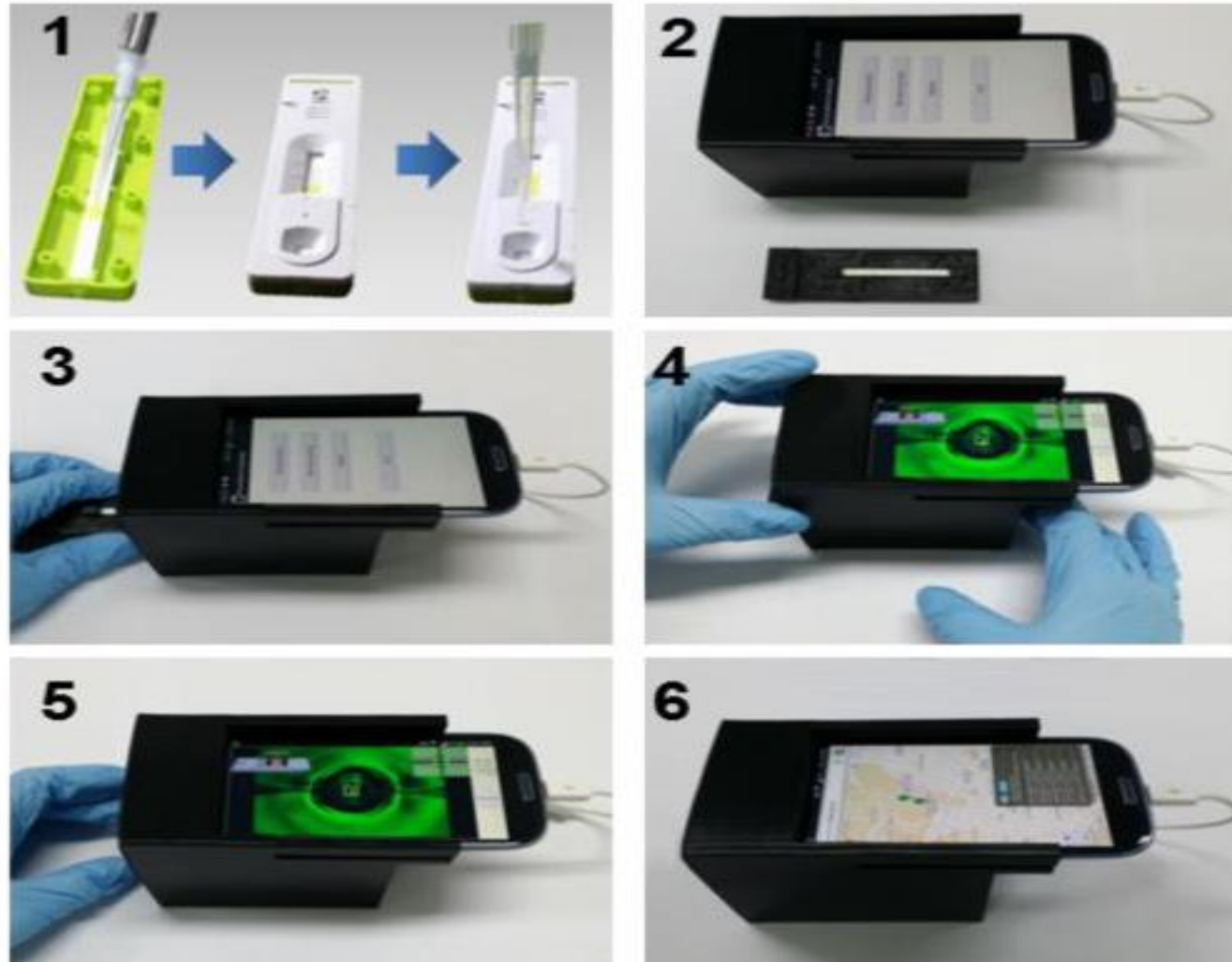
“Stand-Alone” Bütünleşik Sistemler ; eSensor (GenMark)

FDA onaylı solunum yolu paneli,
HCV RNA ve Genotiplendirme
kiti yolda



Research Paper

Smartphone-Based Fluorescent Diagnostic System for Highly Pathogenic H5N1 Viruses

Seon-Ju Yeo^{1‡}, Kyunghan Choi^{2‡}, Bui Thi Cuc¹, Nguyen Ngoc Hong¹, Duong Tuan Bao¹, Nguyen Minh



Lab-on -Chip



Point of Care Tests (Yerinde Bakım Testleri)

«POC testing as patient specimens being assayed at or near the patient, with the assumption that test results will be available instantly or in a very short time frame, to assist care-givers with immediate diagnosis and/or clinical intervention.»

POC testlere olan gereksinimler sağlık altyapıları güçlü ve herkes için ulaşılabilir olan gelişmiş ülkelerle sağlık altyapısı zayıf ve çoğunlukla erişilebilir tek test seçeneği olan az gelişmiş ülkelerde farklı

Kolay uygulanabilir olmakla birlikte POC testler geçmişte genel olarak:

- Teknisyen hatalarına açık
- Duyarlılık ve özgüllükleri düşük

Affordable

Sensitive

Specific

User-friendly

Rapid

Equipment-free

Delivered to those in need

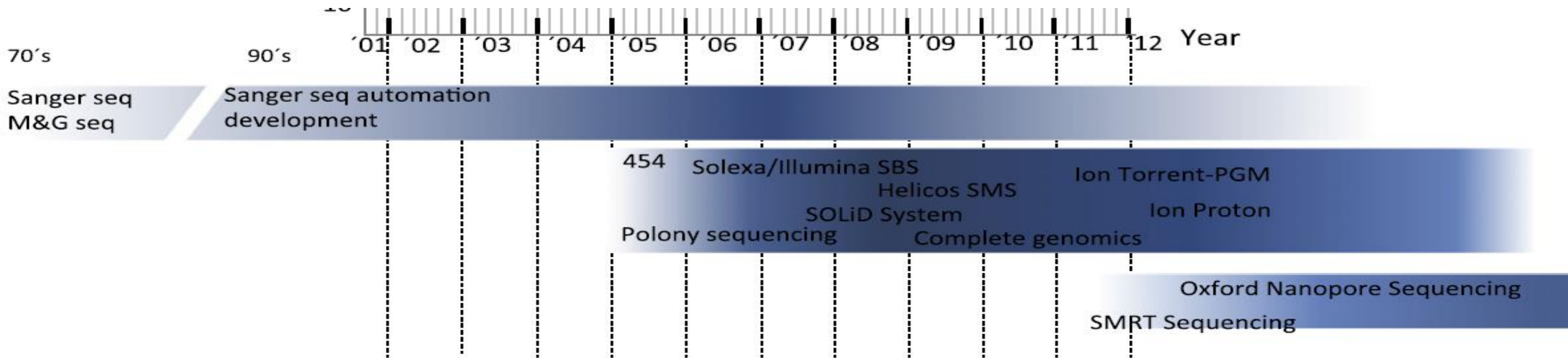
Point of Care Tests (Yerinde Bakım Testleri)

- Toplumda kullanımı???
- Eczanelerde test yapılması
- Kendi kendine testler → Evde testler
- Mobil teknolojiler
- Sahada mobil teknolojiler

“Point of care” Test örnekleri

Platform	Üretici	Örnek Hazırlama	Amplifikasyon Yöntemi	Saptama Yöntemi	Test Süresi (Dakika)
GeneXpert	Cepheid	Evet	PCR	Gerçek Zamanlı Fluoresans	90-120
Liat Analyzer	IQuum	Evet	PCR	Gerçek Zamanlı Fluoresans	<60
FilmArray	IdahoTechnolgies	Evet	PCR	Son Fluoresans Ölçümü	60
LA-120	Eiken	Hayır	LAMP	Gerçek Zamanlı Fluoresans	<60
Twista	TwistDx	Hayır	RPA	Gerçek Zamanlı Fluoresans	<20
GenieII	Optigene	Hayır	LAMP	Gerçek Zamanlı Fluoresans	<20
BESTCassette	U-StarBiotech BioHelix	Hayır	İzotermal	Lateral Flow	Bilinmiyor
Simplexa Direct Amplification Disc	Focus 3M Integrated Cyclor	Evet	PCR	Gerçek Zamanlı Fluoresans	60

Yeni Kuşak Dizileme Sistemleri

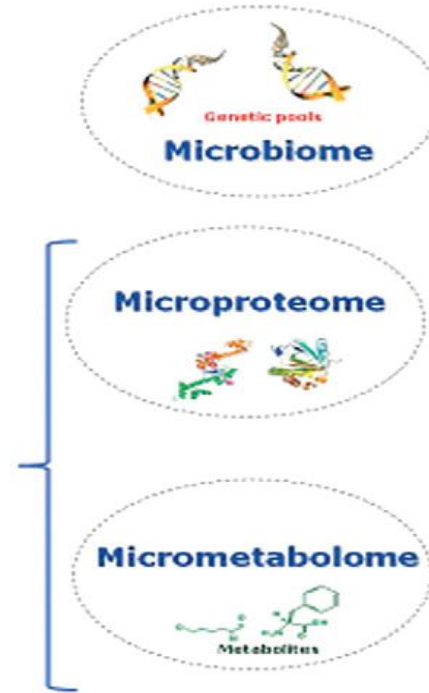
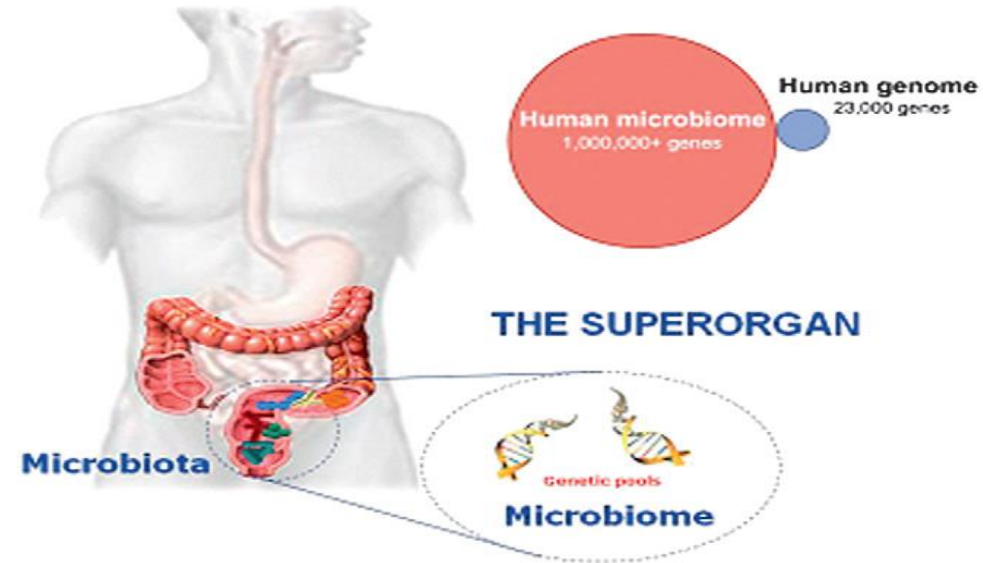


Temel Yeni Kuşak Dizileme Uygulamaları

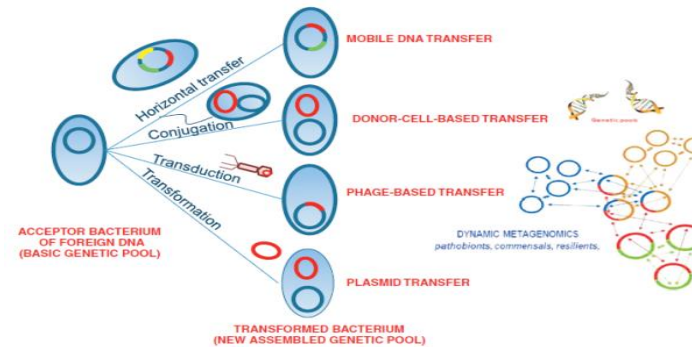
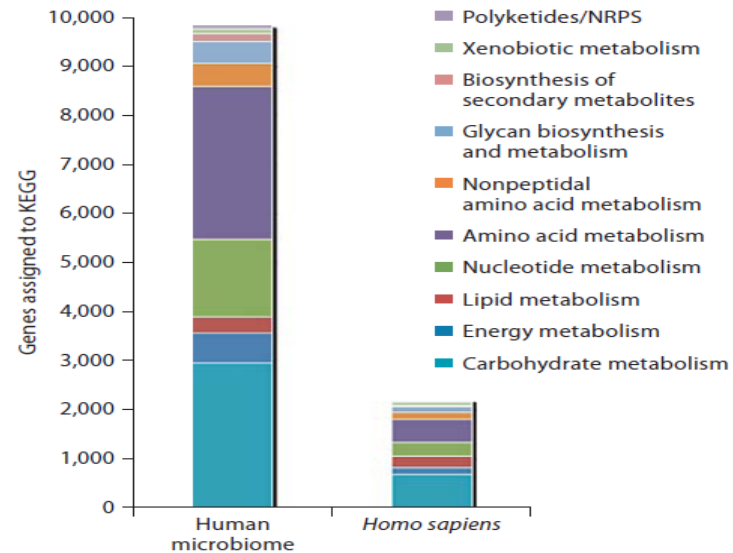
- Metagenomik yaklaşımlar ve patojen tanımlama;
- Patojenin konakla ve konaklar arası etkileşiminin incelenmesi (Salgın analizi, moleküler epidemiyoloji);
- Konak-etken ilişkisi çalışmaları (İlaç direnci, aşı ve yeni tedavi yöntemlerinin geliştirilmesi)
- Konağın genetik yapısının incelenmesi (infeksiyonlara yatkınlık, direncin anlaşılması → kişiselleşen tıp)

Metagenomik Yaklaşım

THE SUPERORGANISM



Mass sequencing



Yeni Kuşak Dizileme Yöntemleri

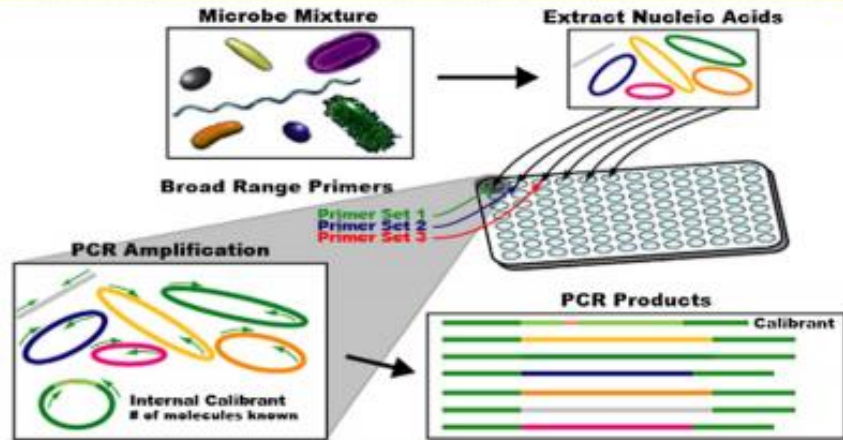
Zayıf Halka = Biyoinformatik



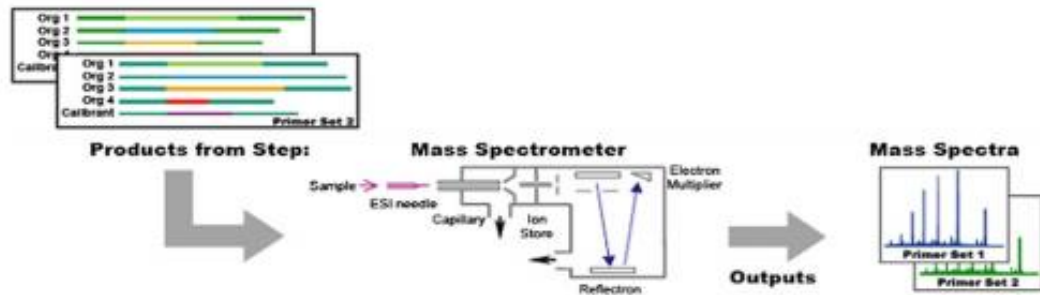
Kütle Spektrofotometresi:

PCR Coupled to Electrospray Ionization Mass Spectrometry (PCR/ESI-MS)

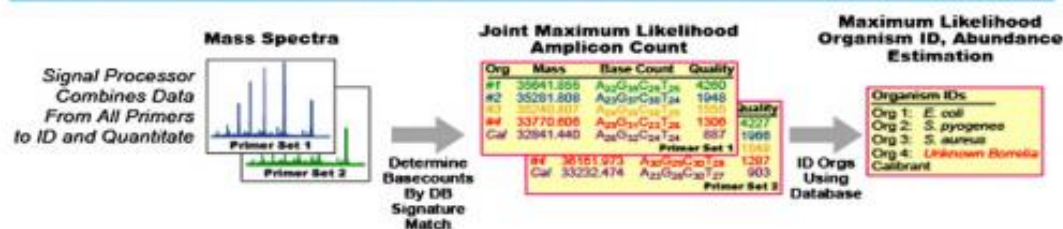
STEP #1 - DNA extraction and PCR with universal primers and calibrant plasmids



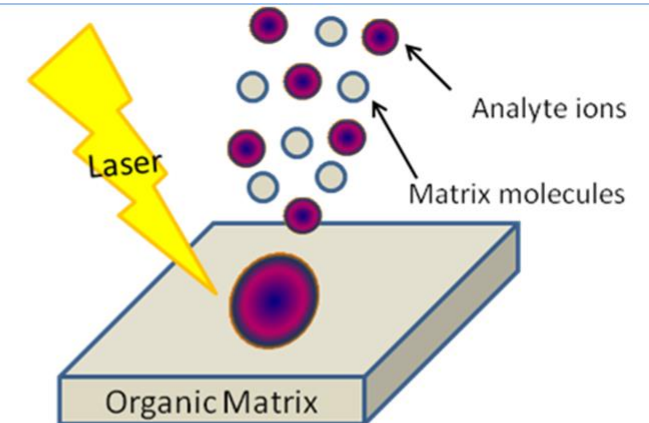
STEP #2 - Electrospray products into mass spectrometer



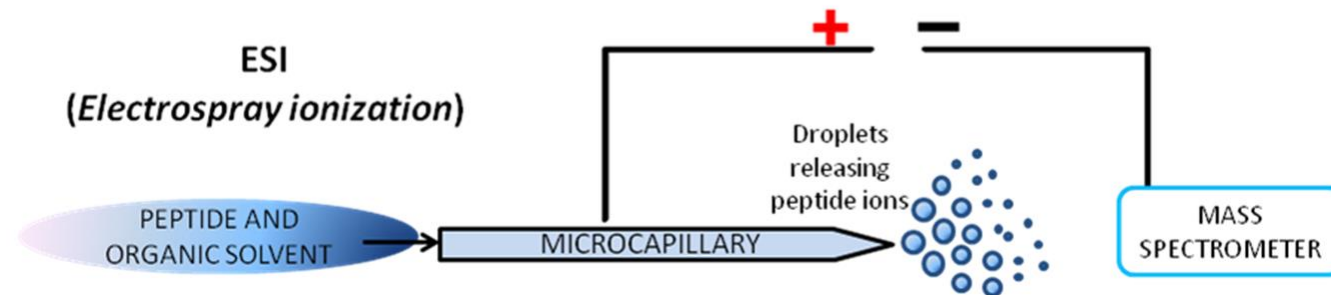
STEP #3 - Signal Processing - ID organism using Mass Spec data from multiple primers



A) MALDI (Matrix-assisted laser desorption/ionization)



B) ESI (Electrospray ionization)



Kütle Spektrofotometresi:

PCR Coupled to Electrospray Ionization Mass Spectrometry (PCR/ESI-MS)

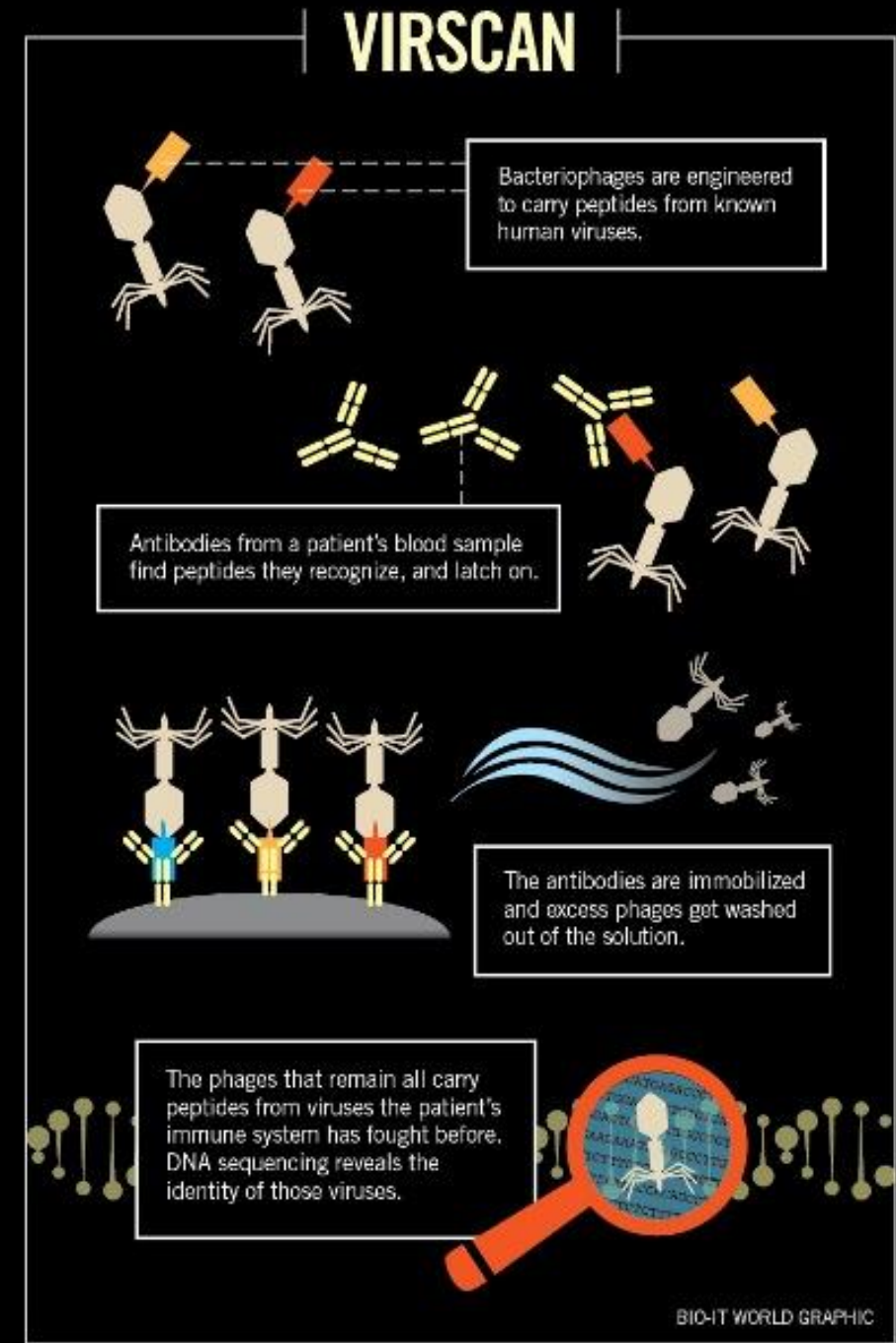
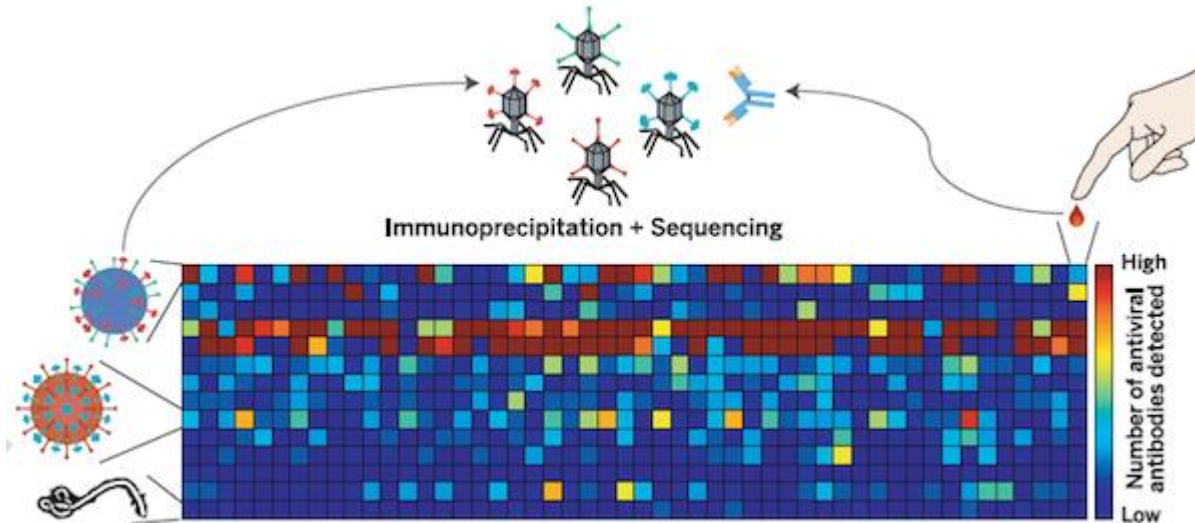
ASSAY	COVERAGE	SAMPLE TYPE
BAC BSI BAC SFT	750+ Bacteria , Candida and 4 Antibiotic Resistance Markers: mecA, vanA, vanB and kpc	5ml EDTA whole blood Sterile fluids and tissues
BAC LRT	Identical coverage as BAC BSI and BAC SFT with additional semi-quantitative threshold	BAL and ETA
Fungal	200+ fungi	BAL and Isolates
Viral IC	130+ viruses in 13 reporting groups	Plasma

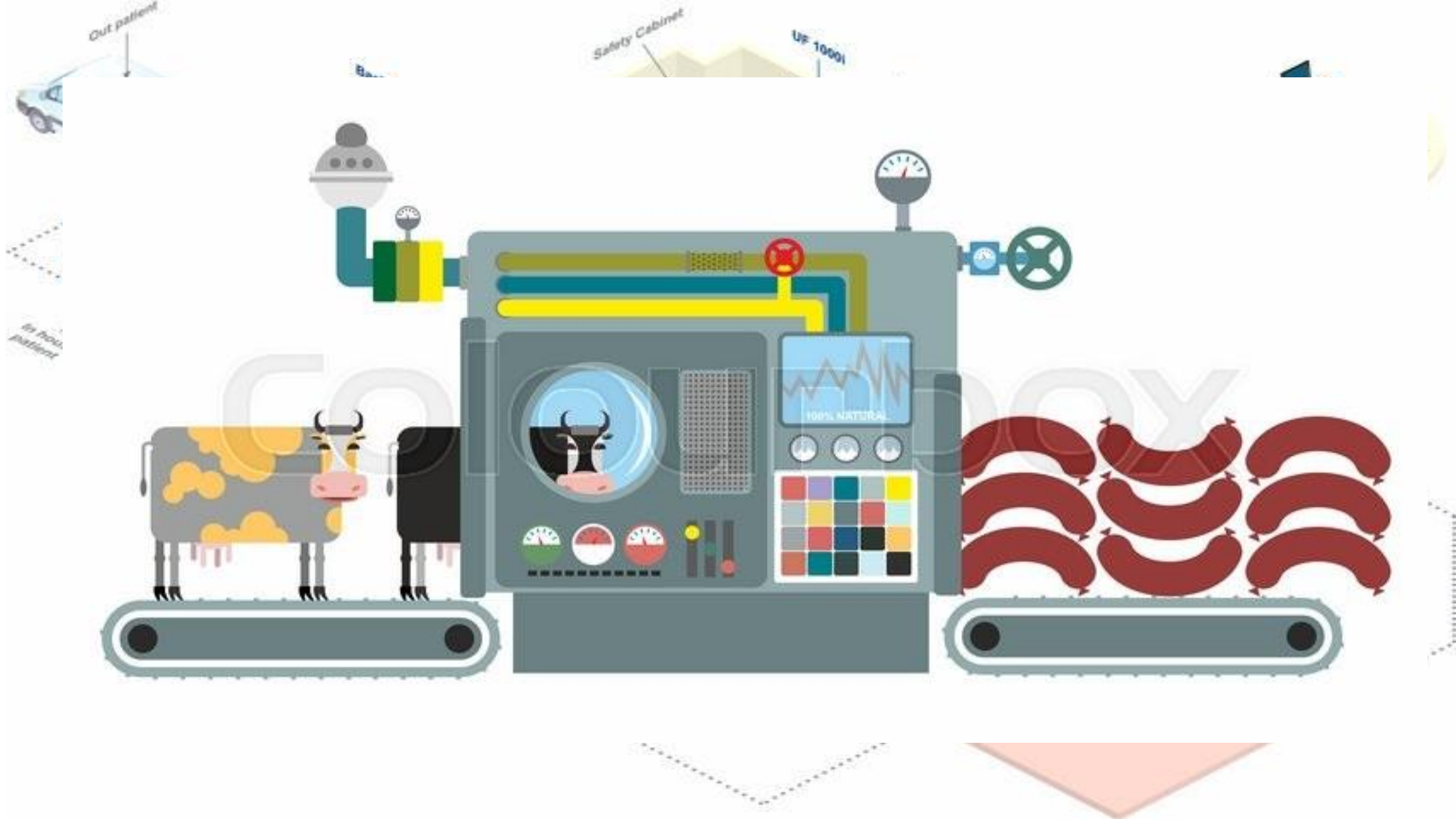
CE Marked. For In Vitro Diagnostic Use.

“Phage Display”

93,000 Kısa DNA fraagmanı
206 virus türüne ait
1,000 bilinen insan virus
kökenini tanıyabilme

\$25

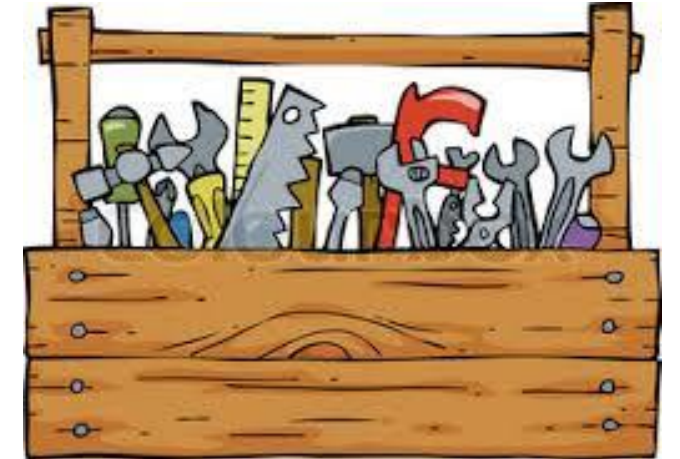
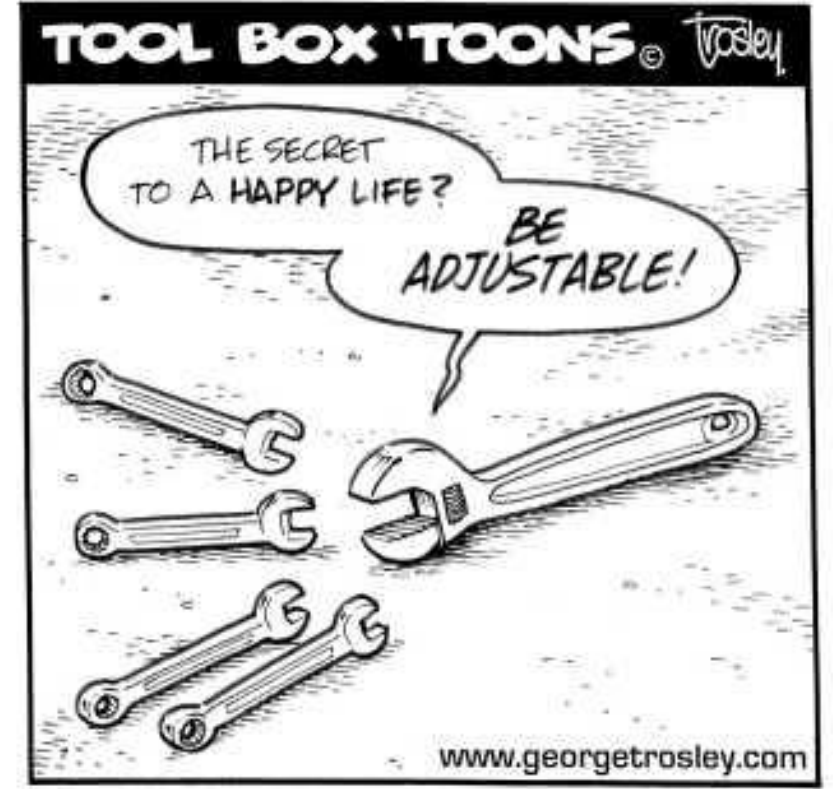




YENİ BİR ÇAĞ



Alet Çantasındaki Pek Çok Alet



Lean Microbiology-Removing «Muda»

Artı Değer Üretmeyen Etkinliklerin Sonlandırılması

- Tip 1 Muda: «İşe yaramıyorsa yapma» yaklaşımı ile hemen elimine edilebilecekler.
- Tip 2 Muda: İşlerin şu an için yapılma şekli için gerekli olan ve elimine edilemeyecekler (denetim, gözetim, kalite kontrol için gerekli olanlar...)

*the microbiology laboratory today is exceedingly “faced with a **superabundance of academic information** and pressure to perform **exhaustive, expensive, clinically irrelevant** [testing]”, which, when misguided “misleads physicians into erroneous diagnosis and inappropriate therapy”.*

“more practical, economical, clinically meaningful approach”



Hastam
infektemi ?

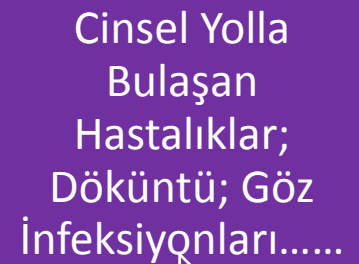
Temel Beklentiler;



Etken Ne ?



Etkin
Antimikrobiyal
Ajan Hangisi ?



Sendromik Multipleks Tanı Kitleri

Bakteriyel, Viral, Fungal, Parazitel Etkenler + Direnç

To Be or Not to Be...

SELF-EMPLOYED



HEDEF

Doğru Test

Doğru Hasta

Doğru Zaman

Temel
Soru

Mevcut klinik
durum ve fiziki
şartlar için
uygulanabilir
bir test mi?

Bu testin
hastaya (ve
hastalara)
faydası var mı?

Test sonucu
kullanılabilecek
kadar hızlı elde
edilecek mi?

HEDEF

Doğru Test

Doğru Hasta

Doğru Zaman

Temel
Sorular

Duyarlılık / Özgünlük → “gold standard”

Prediktif Değerler → “ruling out”

Tanı Değeri

Test Hacmi → Tek tek- Toplu çalışma??

Laboratuvar / Hastane Altyapısı → POCT

Maliyet

Klinik/Toplam Etki

HEDEF

Doğru Test

Doğru Hasta

Doğru Zaman

Temel
Soru

Endikasyon Seçimi
Uygun kullanım kriterleri
Öncelik tanımları Tek tek- Toplu çalışma
Refleks test & Örnek Reddi
Bilgisayar Destekli İstem “computerized order
entry” (CPOE)

HEDEF

Doğru Test

Doğru Hasta

Doğru Zaman

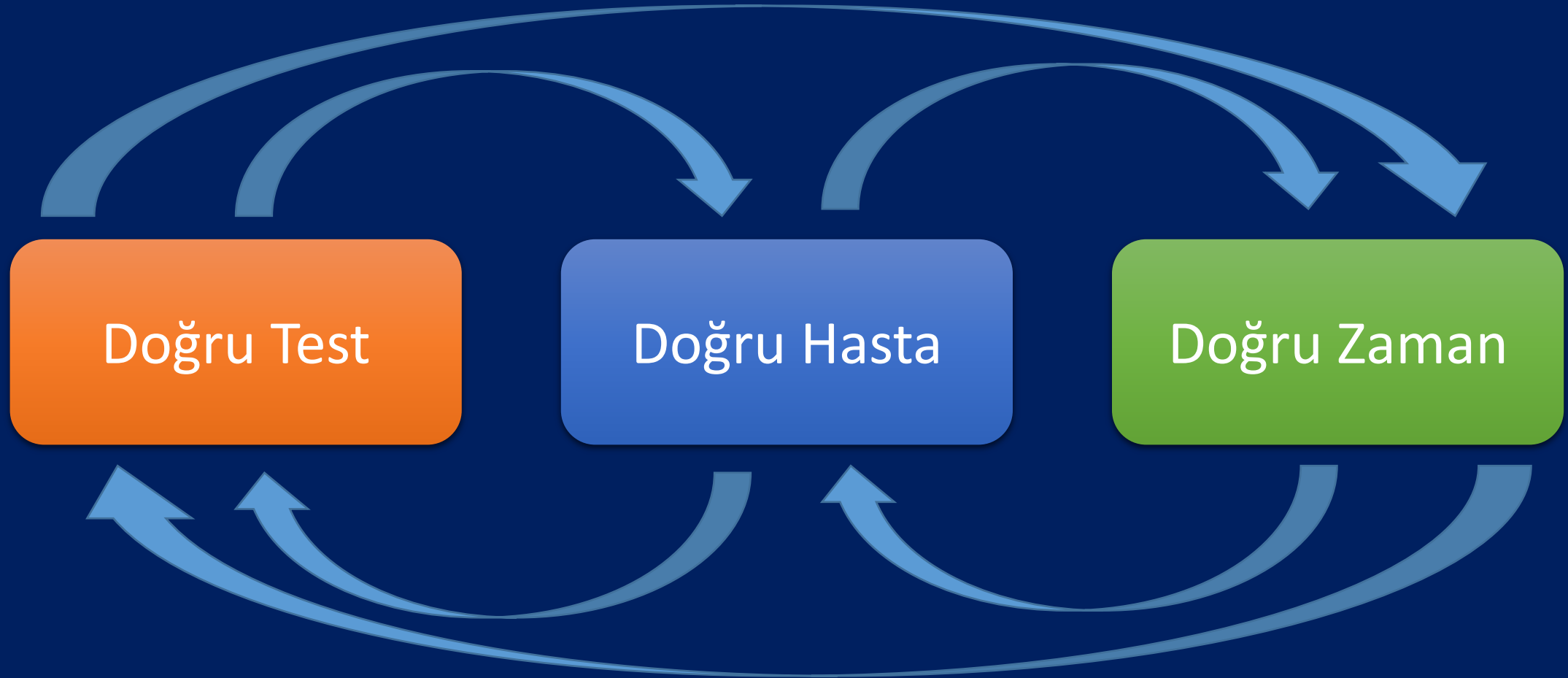
Temel
Soru

Örnek alım yeri ve zamanlaması
Merkezi ya da POCT
Tek tek ya da toplu çalışma
Sonuç Çıkma süresi

Doğru Test

Doğru Hasta

Doğru Zaman



Maliyet Etkinlik

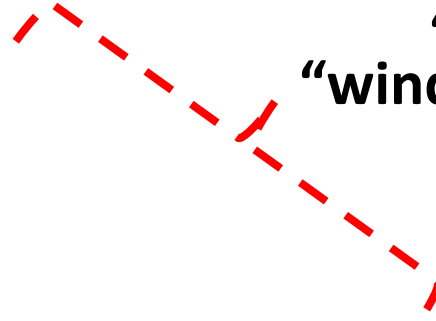
			SUT
4008	900.200	Alanin aminotransferaz (ALT)	1,09 ₺
4009	900.210	Albümin	0,99 ₺
4021	900.340	Alkalen fosfataz	1,09 ₺
4025	900.370	Amilaz	1,39 ₺
4047	900.580	Aspartat transaminaz (AST)	0,99 ₺
4059	900.690	Bilirubin Direkt	0,99 ₺
4059	900.690	Bilirubin Total	0,99 ₺
4081	900.901	CRP, nefelometrik	4,48 ₺
4089	901.020	Demir (Serum)	1,09 ₺
4091	901.040	Demir bağlama kapasitesi	1,09 ₺
4114	901.220	Ferritin	4,97 ₺
4119	901.260	Fosfor (P)	0,99 ₺
4141	901.500	Glukoz	0,99 ₺
4164	901.730	İdrar mikroskobisi	1,79 ₺
4168	901.780	TİT	4,97 ₺
4182	901.910	Kalsiyum (Ca)	1,09 ₺
4199	902.090	Klor (Cl)	0,99 ₺
4208	902.180	Kreatin	1,09 ₺
4209	902.190	Kreatin kinaz (CK)	1,39 ₺
4212	902.220	Kreatinin klerens testi	3,38 ₺
4217	902.260	Laktik Dehidrogenaz (LDH)	0,99 ₺
4296	903.130	Potasyum	1,09 ₺
4300	903.170	Procalcitonin	25,37 ₺
4323	903.400	Sedimentasyon	1,69 ₺
4330	903.470	Serbest T3	4,48 ₺
4331	903.480	Serbest T4	4,48 ₺
4153	901.620	Tam Kan (Hemogram)	2,98 ₺
		Toplam	77,01 ₺

4871	908.732	Reverse Transcriptase PCR Multiplex	218,89 ₺
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Hastam
İnfektemi ?

Örnek Toplama

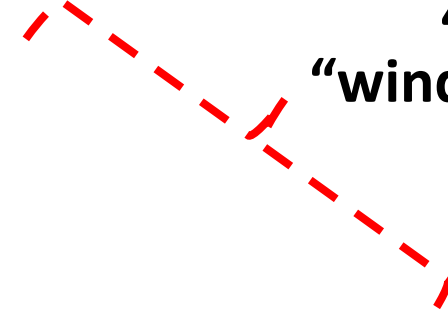


ZAMAN
“window period”



Etken Ne ?

Organizma Tanımlama



ZAMAN
“window period”



Etkin
Antimikrobiyal
Ajan Hangisi ?

**Antimikrobiyal Duyarlılık
Testi**

Klinik Değerlendirme

POCT

İnfeksiyon Kontrolü

HASTA

KLİNİK

İNFEKSİYON
HASTALIKLARI

MİKROBİYOLOJİ
LABORATUVARI

Tanı ve
Tedavi

Antimikrobiyal
Yönetim:
*Doğru Değerlendirme
*Doğru Antimikrobiyal
*Doğru Zaman

Test Sonucu
Raporlanması

Tanı Yönetimi:

- *Doğru Test
- *Doğru Hasta
- *Doğru Zaman
- *Doğru Yer

Moleküler Tanı
Testi İstemi

Impact of a Healthcare Provider Educational Intervention on Frequency of *Clostridium difficile* Polymerase Chain Reaction Testing in Children: A Segmented Regression Analysis

Larry K. Kociulek,^{1,5} Maria Bovee,² Donna Carter,³ Jody D. Ciolino,⁶ Rupal Patel,⁴ Anna O'Donnell,² Angela H. Rupp,² Xiaotian Zheng,^{3,7} Stanford T. Shulman,^{1,5} and Sameer J. Patel^{1,5}
¹Pediatric Infectious Diseases, ²Infection Prevention and Control, ³Pathology and Laboratory Medicine, ⁴Department of Pharmacy, Ann & Robert H. Lurie Children's Hospital of Chicago, ⁵Departments of Pediatrics, ⁶Preventive Medicine-Biostatistics, and ⁷Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois

Results. Hospital-wide, absolute TR reduction was 0.71 ($P[\text{level}] = .0067$; $P[\text{trend}] = .0042$) and absolute PR reduction was 0.14 ($P[\text{level}] = .22$; $P[\text{trend}] = .018$). In the outpatient setting, absolute TR reduction was 0.30 ($P[\text{level}] = .0015$; $P[\text{trend}] < .001$) and absolute PR reduction was 0.09 ($P[\text{level}] = .0069$; $P[\text{trend}] = .046$). The incidence density of healthcare facility-associated CDI did not significantly change after the EI. The EI was associated with avoidance of 574 tests and 113 positive tests (and subsequent antibiotic courses) during the postintervention period, which saved approximately \$250 000 in patient charges related to CDI testing and treatment.

Table 1. Topics Included in the Healthcare Provider Didactic Education

Topics Included in 15-Minute Clinician Didactic Education
• Epidemiology of <i>Clostridium difficile</i> infection (CDI) and asymptomatic carriage
• <i>C difficile</i> polymerase chain reaction test interpretation
• American Academy of Pediatrics recommendations for CDI testing [19]
• Hospital CDI surveillance and <i>C difficile</i> testing data
• Impact of CDI misdiagnosis on patient care and hospital CDI surveillance
• Suggestions for improving CDI testing behaviors
• Questions and answers
Additional Topics Included in 30-Minute Microbiology Technologist Didactic Education
• Review of criteria for rejecting specimens for CDI testing
• Guidance for responding to healthcare provider inquiries after specimen rejection

Table 2. Electronic Medical Record Prompt When Ordering *Clostridium difficile* Polymerase Chain Reaction (PCR) Testing

Because <i>C difficile</i> PCR is highly sensitive and frequently identifies colonized patients, testing should NOT be ordered for patients with low probability of infection, such as the following:
• A patient without risk factors who has vomiting as a significant complaint.
• The stool is soft or formed.
• A patient has diarrhea and is prescribed stool softeners or laxatives.
• The test is ordered as a “test of cure” after treatment.
• A negative <i>C difficile</i> PCR result was reported within the last 7 days.

Genişletilmiş Spekturumlu Yönetim

A.I.D. + C

Antimicrobial Stewardship

–

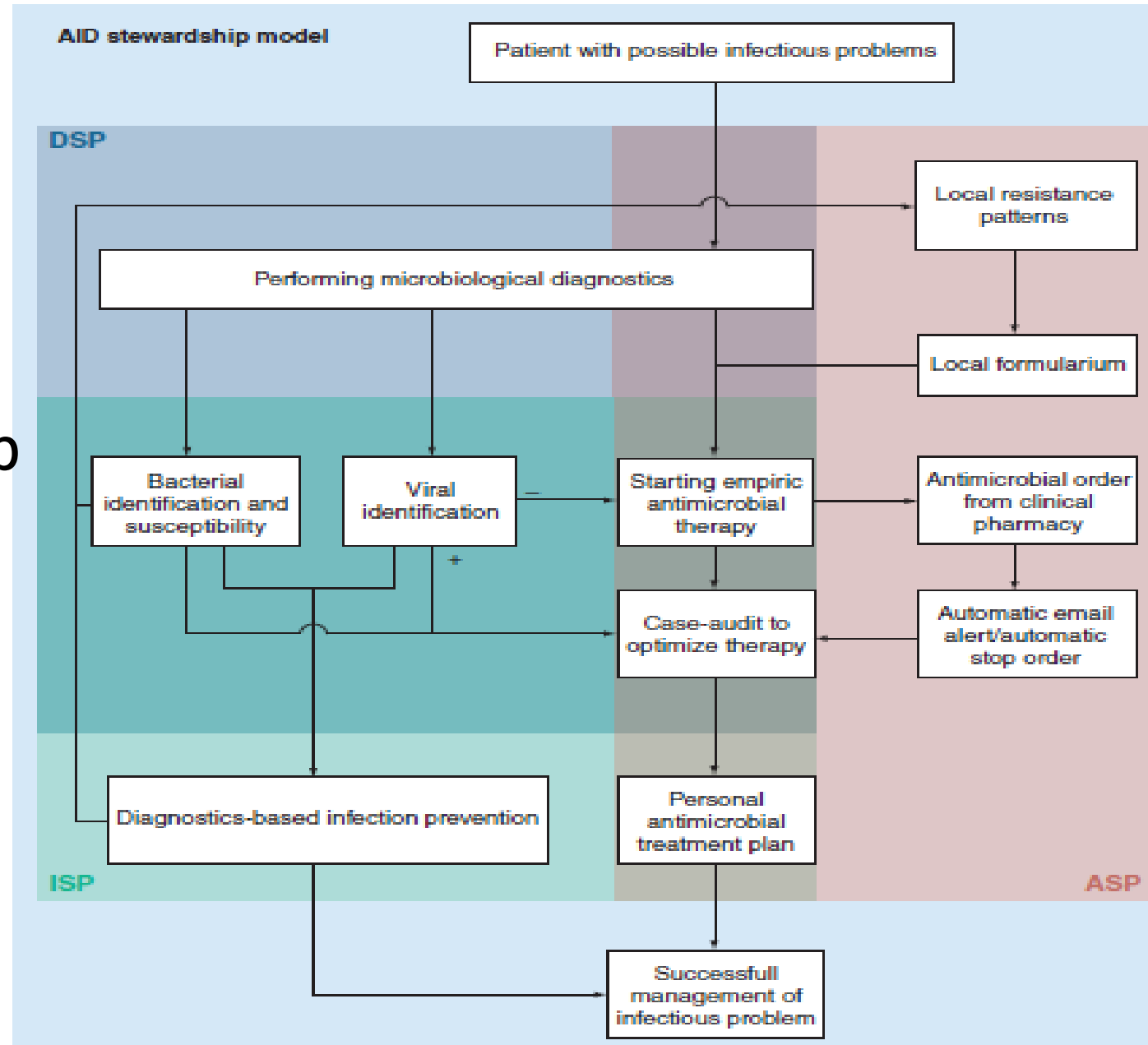
Infection Control Stewardship

–

Diagnostic Stewardship

+

Cost Stewardship



Kültüre (ve mikroskopiye) dayalı bir kültür....



cannot currently, and perhaps will never be able to, provide a definitive 'no' to rule-out infection

Messacar K. ve ark. J. Clin. Microbiol. doi:10.1128/JCM.02264-16

Teşekkürler...