25. TÜRK KLİNİK MİKROBİYOLOJİ VE İNFEKSİYON HASTALIKLARI KONGRESİ

KLİNİK MİKROBİYOLOJİDE YENİ NESİL DİZİLEME KURSU

Enfeksiyon Hastalıklarında Yeni Nesil Dizileme Önemi

Dr. Mert Ahmet KUSKUCU

KOÇ ÜNİVERSİTESİ TIP FAKÜLTESİ

KUISCID KOÇ UNIVERSITY - İŞBANK CENTER FOR INFECTIOUS DISEASES

MİBİTÇG

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Date Range

2019-12-21 2020-05-15

PAUSE RESET

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Phylogeny

ZOOM TO SELECTED RESET LAYOUT

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19A 19B 20A 20C 20D

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Date

Geography

RESET ZOOM

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Nucleotide diversity of genome

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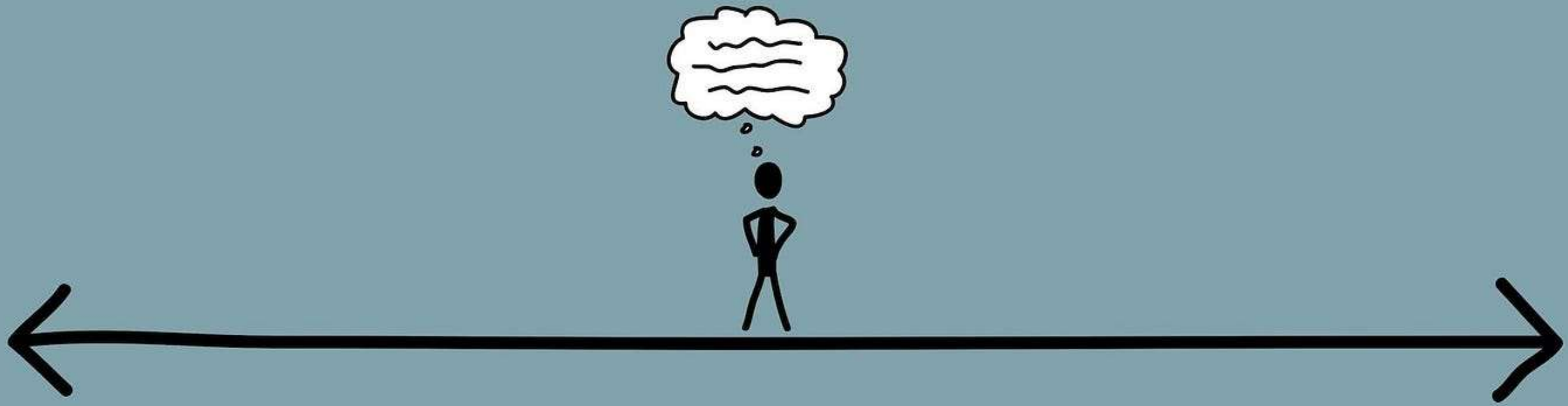
ORF1a ORF1b S M N

Infeksiyon Hastalığı Tanı



Ne Yapıyorum ?

Ne Yapardım ?

Ne Yapacağım ?

Infeksiyon Hastalığı Tanı;
Ne Yapardım, Ne Yapıyorum, Ne Yapacağım?

	Yöntem	Avantajları	Dezavantajları
Klasik Yaklaşım	Boyama Yöntemleri (Gram, EZN vb)	Hızlı Ucuz	Yüksek etken yükü olmadıkça düşük duyarlılık Düşük özgüllük
	Kültür Yöntemleri	Çok sayıda örnek çalışmaya uygun Ucuz Altın Standart	Antibiyotik ve antifungal kullanımıyla sınırlı duyarlılık Hassas organizmalar için sınırlı hassasiyet Viral testte sınırlı kullanım Özellikle aside dirençli bakteri ve mantar kültürlerinde sonuç almak için uzun zaman gerekli
	Serolojik Yöntemler	Akut enfeksiyondan sonra tanı potansiyeli Ucuz	Akut enfeksiyon sırasında negatif olabilir Humoral bağışıklık yetersizliklerinde yanlış negatifler Yanlış pozitifler

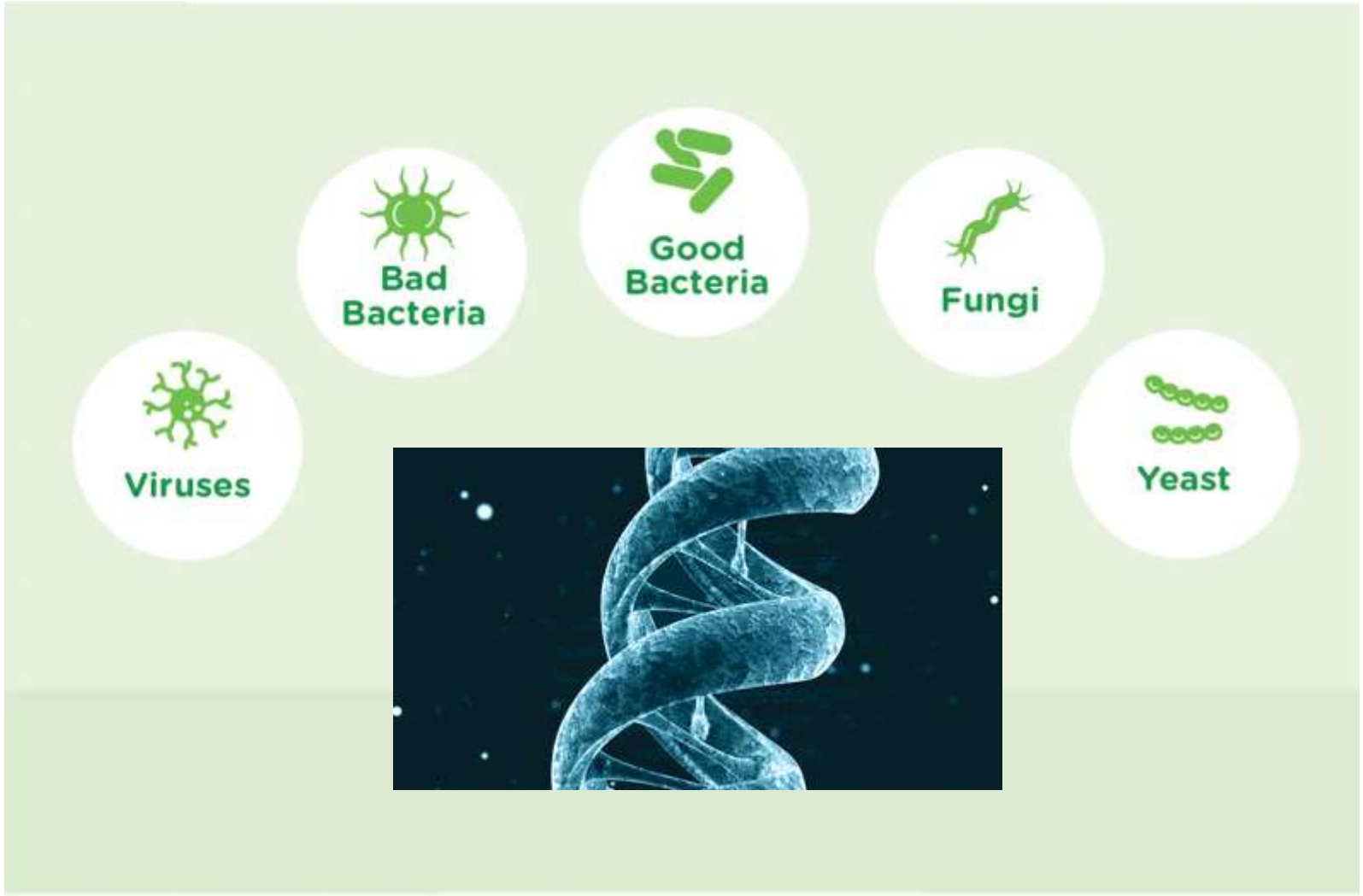
Infeksiyon Hastalığı Tanı;
Ne Yapardım, Ne Yapıyorum, Ne Yapacağım?

	Yöntem	Avantajları	Dezavantajları
Yeni Klasikler	Hedefe Yönelik PCR	Basit Hızlı Ucuz Kantitatitasyon yapabilme	**Hipoteze bağlıdır** Her zaman çalışmayabilecek primerler Genomun çok küçük bir kısmıyla sınırlıdır
	Çoklu PCR	Hızlı Birden fazla organizmayı tespit edebilir	Birden fazla amplicon içerdiği için görece olarak tek hedefli PCR'lara göre düşük duyarlılık Primer bağlanma bölgesi mutasyonları nedeni ile yalancı negatiflik
	MALDI-TOF	Yüksek özgüllük Kültür sonrası hızlı	Kültürde üremiş izolat gerektirir

Infeksiyon Hastalığı Tanı;
Ne Yapardım, Ne Yapıyorum, Ne Yapacağım?

	Yöntem	Avantajları	Dezavantajları
Yeni Yaklaşımlar	Klasik Dizileme için Hedefe Yönelik (16s, ITS) Universal PCR	Görece Ucuz Steril vücut sıvısında tek patojeni saptayabilir	Yüksek etken yükü olmadıkça düşük duyarlılık Çevresel kontaminasyon eğilimli
	NGS için Hedefe Yönelik Universal (16s, ITS) PCR	Steril vücut sıvısındaki patojenleri saptayabilir, Çoklu test olasılığı	Yüksek etken yükü olmadıkça düşük duyarlılık Kantitasyon olasılığı Pahalı ve zaman alıcı Kütüphane hazırlamak karmaşık
	Metagenom Analizi	**Hipotezsiz veya tarafsız test** Yeni veya beklenmedik organizmaların keşfi Kantitasyon potansiyeli Genomun herhangi bir bölümünü tespit etme yeteneği Konak ile ilgili bilgi sağlar	Pahalı Zaman alıcı Tüm genomlar için veritabnı mevcut değildir Çevresel kontaminasyon eğilimli

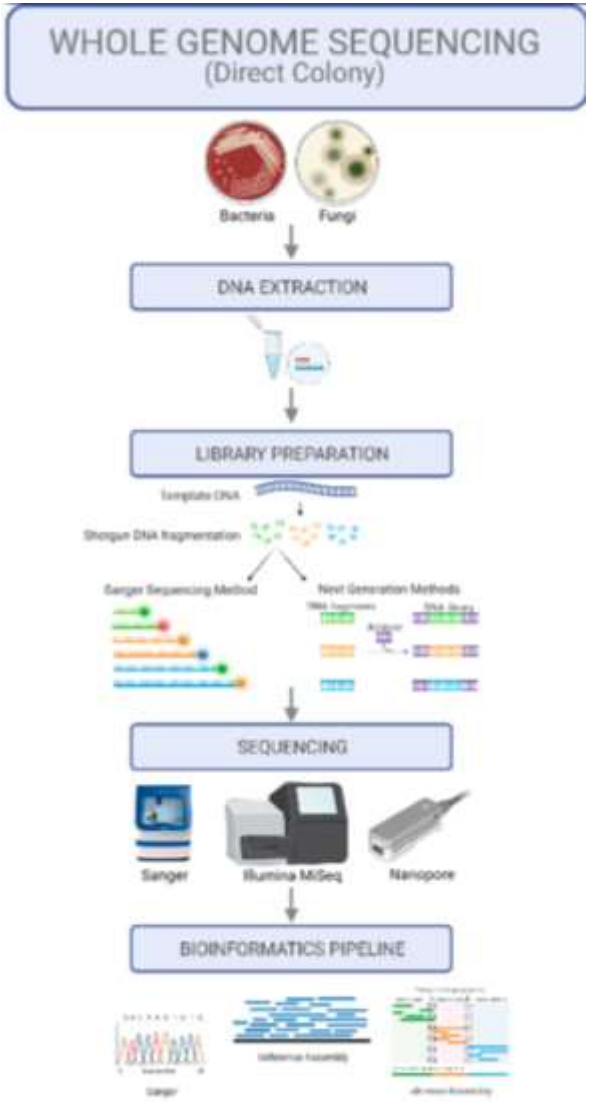
Yeni Nesil Dizileme,
Genomik Çağda Enfeksiyon Hastalıkları Uzmanlığı

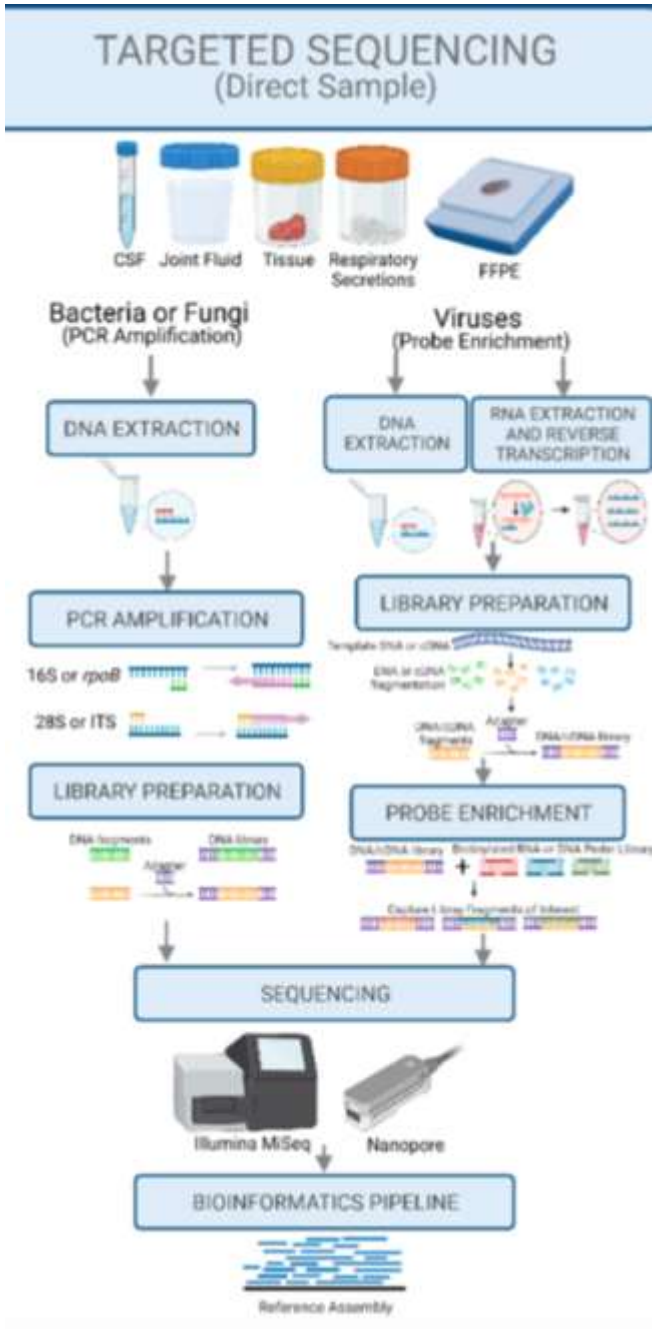


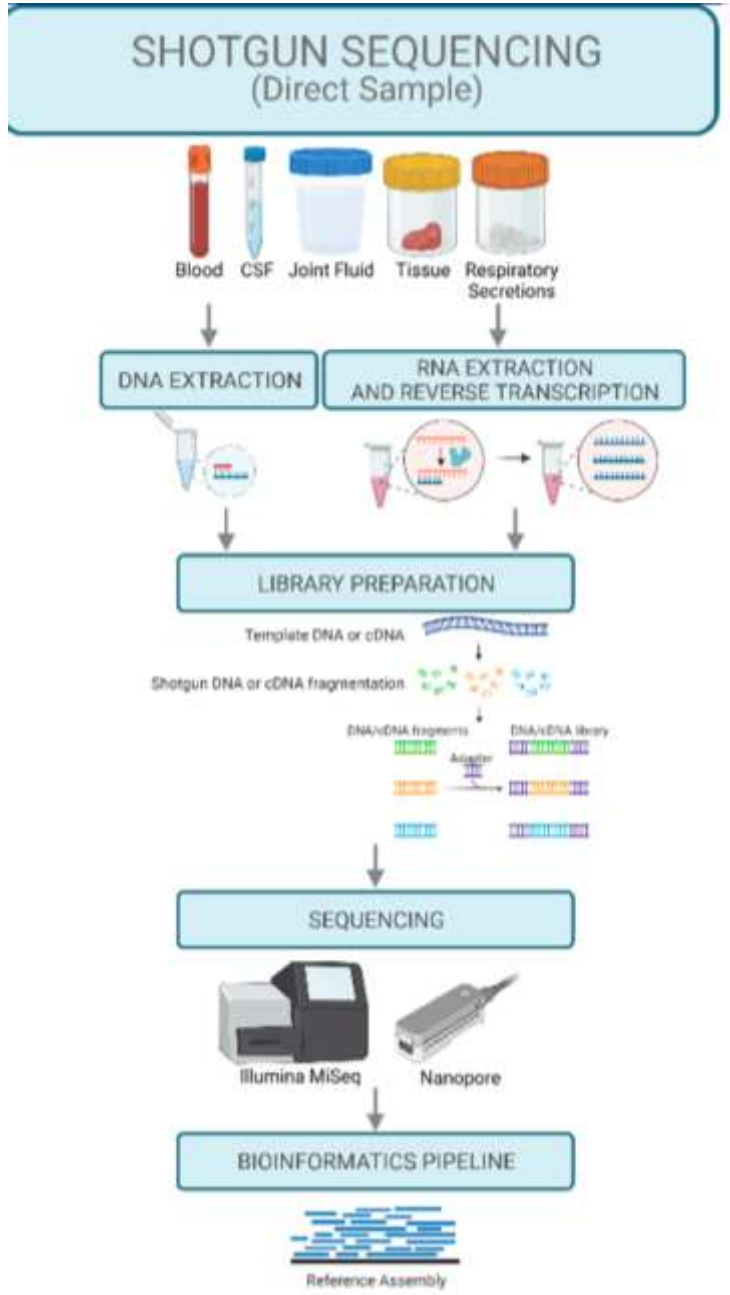
Tarafsız ve Hipotez Olmadan

Hypothesis-Free !!!

Tarafsız ve Hipotez Olmadan

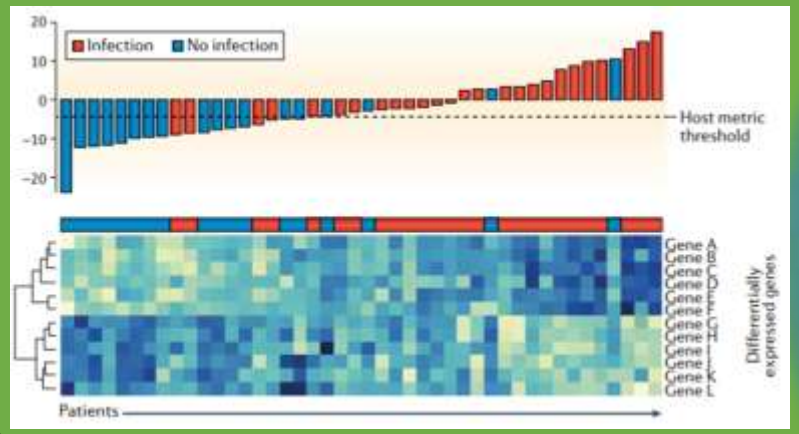






Metagenomik Tanı Yaklaşımı

İnsan Genomu



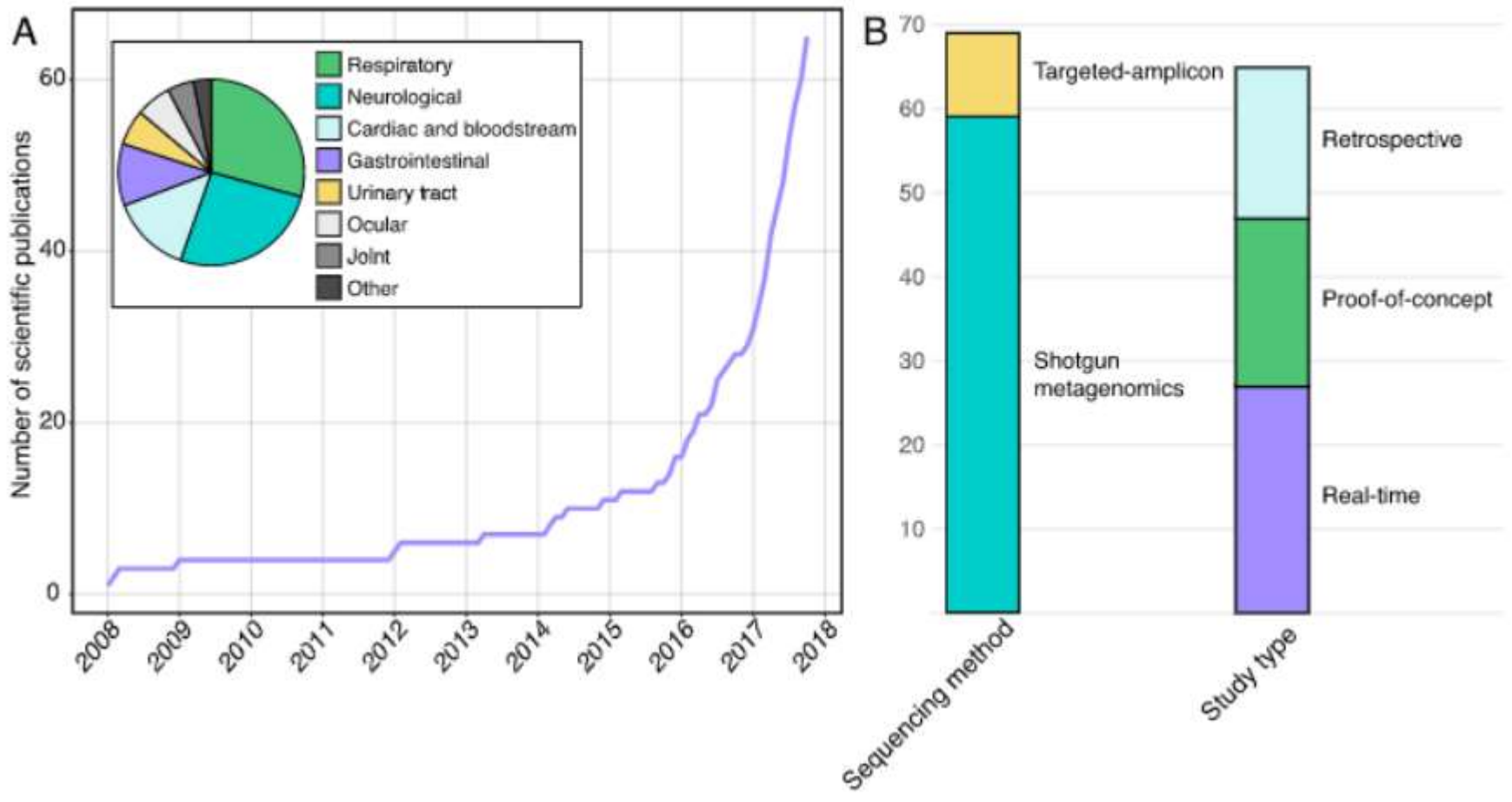
Steril Örnekler

Floralı Örnekler

Mini Review

Highlighting Clinical Metagenomics for Enhanced Diagnostic Decision-making: A Step Towards Wider Implementation

Jessica D. Forbes [a,b,c], Natalie C. Knox [c], Christy-Lynn Peterson [c], Aleisha R. Reimer [c,*]



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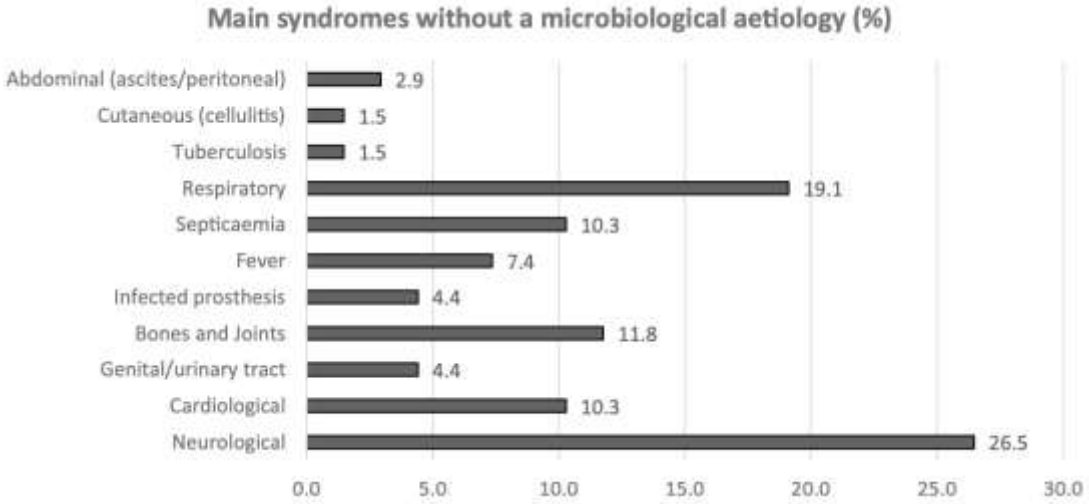
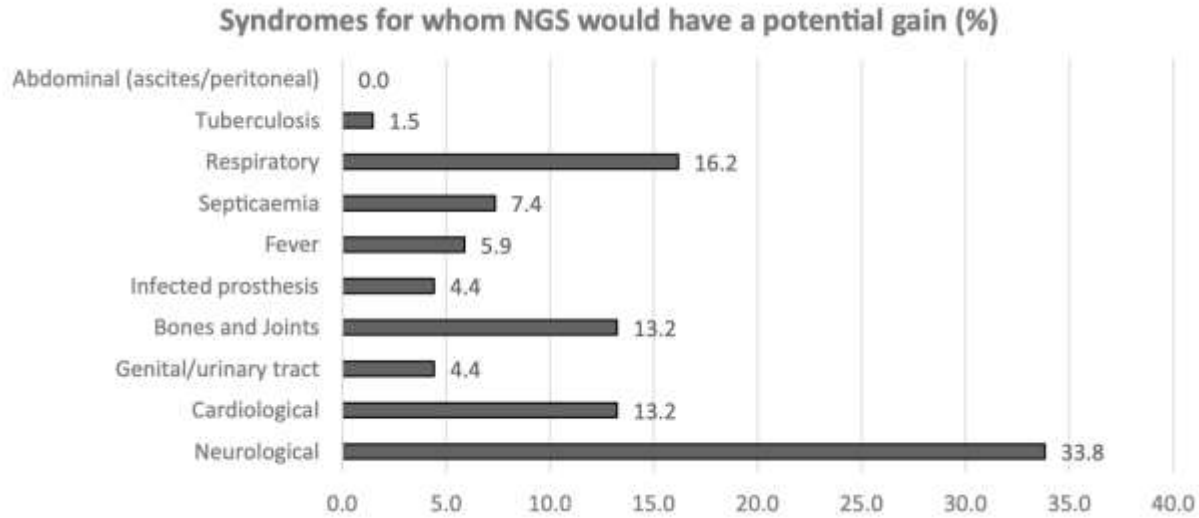


Next-generation sequencing: what are the needs in routine clinical microbiology? A survey among clinicians involved in infectious diseases practice

Charlotte Michel[1*], Charlotte Martin[2], Pierre Smeesters[3,4], Jean-Christophe Goffard[5], Thomas Demuyser[6] and Marie Hallin[7]



Ne Umduk?
Ne Bulduk

Next-generation sequencing: what are the needs in routine clinical microbiology? A survey among clinicians involved in infectious diseases practice

Charlotte Michel[1*], Charlotte Martin[2], Pierre Smeesters[3,4], Jean-Christophe Goffard[5], Thomas Demuyser[6] and Marie Hallin[7]

Background: The translation of Next-Generation Sequencing (NGS) from research to clinical microbiology is increasing rapidly, but its integration into routine clinical care struggles to catch-up. A challenge for clinical laboratories is that the substantial investments made in the required technologies and resources must meet both current and forthcoming needs.

Methods: To get a clinical perspective of these needs, we have sent a survey to infectious diseases clinicians of five hospitals, covering the following topics: NGS knowledge, expected syndromes and patients foreseen to benefit from NGS, and expected impact on antimicrobial prescription.

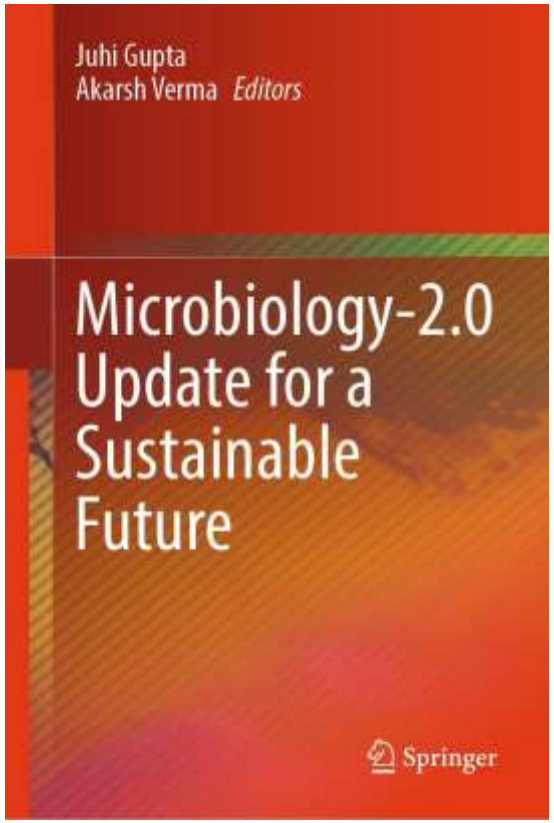
Results: According to clinicians, benefits of NGS are mostly expected in neurological and respiratory infections diagnostics.

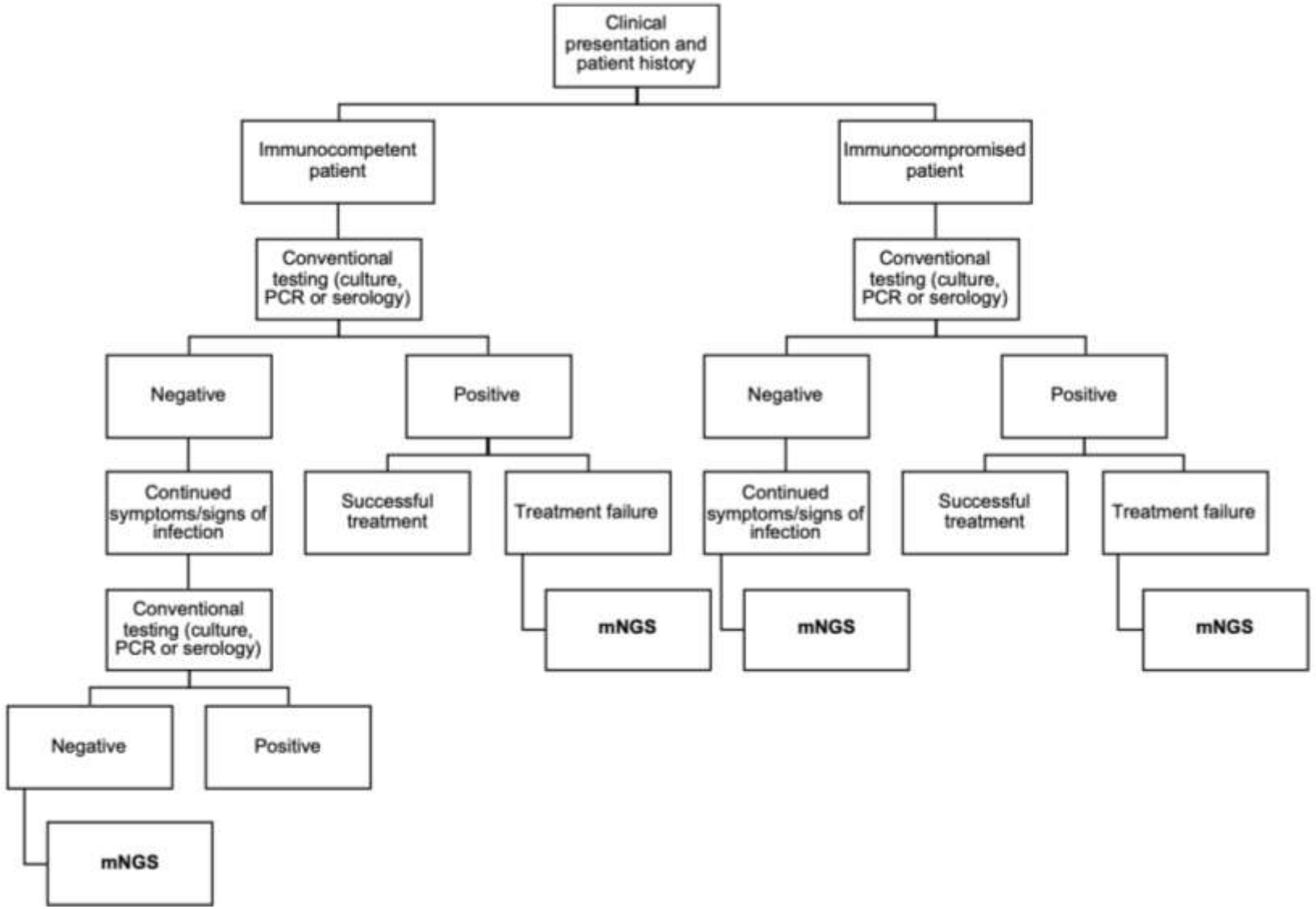
Conclusion: A better dialog between microbiologists and clinicians about hopes and limits of NGS in microbiology may help identifying key investments needed for clinical laboratories, today and tomorrow.

Diagnostic Stewardship for Next-Generation Sequencing Assays in Clinical Microbiology

An Appeal for Thoughtful Collaboration

David C. Gaston, MD, PhD[a,*], Augusto Dulanto Chiang, MD[b], Kevin Dee, MD[b], Daniel Dulek, MD[c], Ritu Banerjee, MD, PhD[d], Romney M. Humphries, PhD[a]





Metagenomics to Assist in the Diagnosis of Bloodstream Infection

Alexander L. Greninger[1*] and Samia N. Naccache[2]

Preanalitik	• Normal cilt florası • Antibiyotik kullamı • Geçici bakteriyemi • mNGS doğrulaması için kullanılan fazla numuneler, steril olmayan kimya veya hematoloji bölümlerinde teknikler
Analitik	• Reaktifler (örn. elüsyon tamponları, nükleik asit ekstraksiyon kitleri, enzimler) sırasında kontamine olmuştur. çevresel viral, bakteriyel veya fungal kirleticilerle üretim • Kullanıcının normal florası veya potansiyel patojenlerin yayılması (gizli solunum veya ishal virüsleri) • Ekstraksiyon, ters transkripsiyon, zenginleştirme, kütüphane hazırlığı sırasında çapraz kontaminasyon • Önceden oluşturulmuş kitaplıklarla çapraz kontaminasyon • Aynı barkod şemasını kullanarak önceki çalışmalardan sızma • Aynı çalıştırmada farklı barkodlardan indeks atlama
Postanalitik	• Bildirilen patojenin kontaminant mı, normal flora mı yoksa kandan gelen bir patojen mi olduğunun belirlenmesi

Chronic Meningitis Investigated via Metagenomic Next-Generation Sequencing

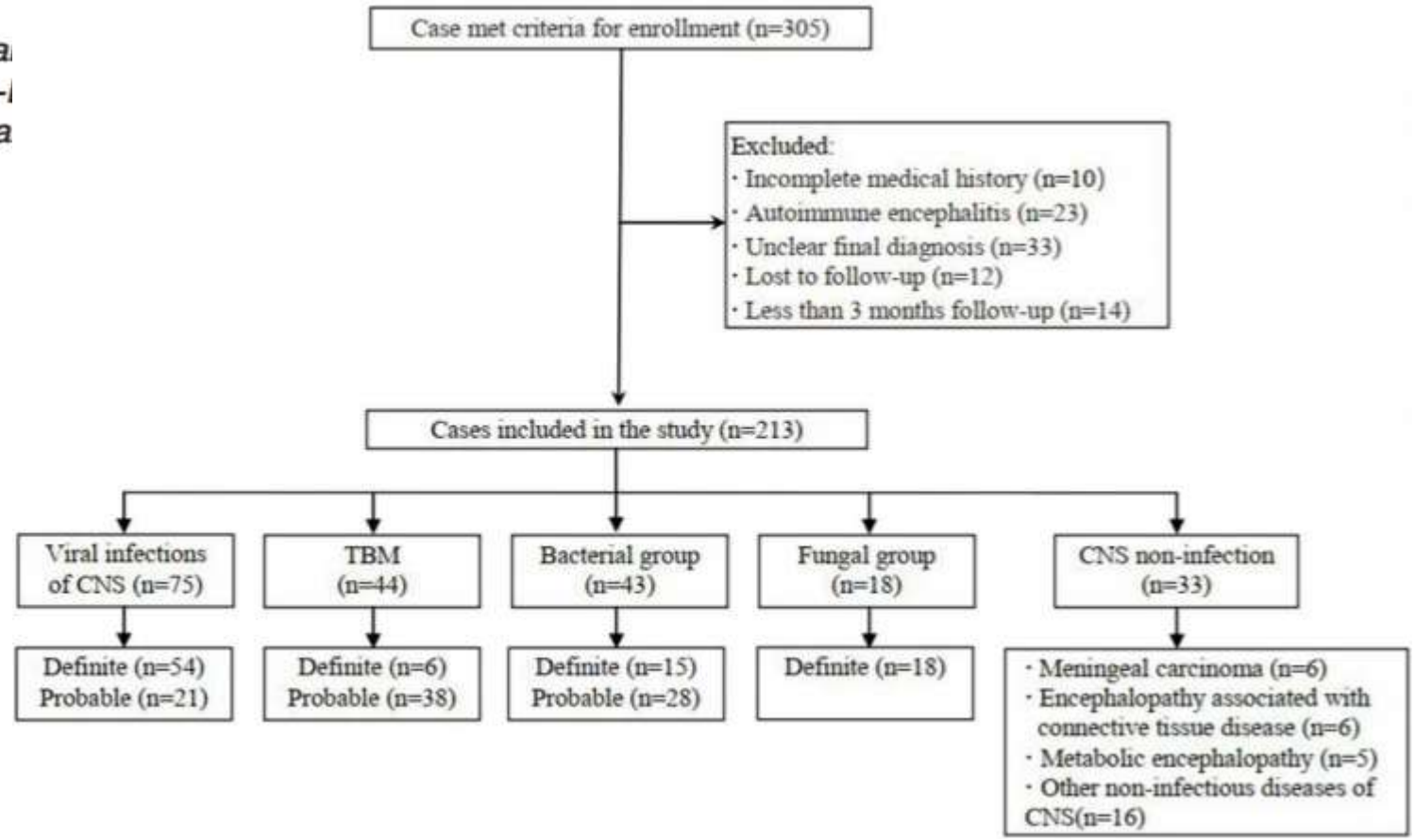
Michael R. Wilson, MD, MAS; Brian D. O'Donovan, MS; Jeffrey M. Gelfand, MD, MAS; Hannah A. Sample, BS; Felicia C. Chow, MD, MAS;

Table 1. Clinical Characteristics of the 7 Participants

Participant No./Sex/Age, y	Final Diagnosis	Clinical Presentation	Disease Duration at Time of Diagnostic LP, mo	Length of Follow-up, mo
1/M/28	*Taenia solium*	Headache, diplopia	11	8
2/F/34	*T solium*	Headache, unilateral facial numbness and tinnitus, recurrent loss of consciousness	9	21
3/M/52	*Cryptococcus neoformans*	Seizures, coma	3	19
4/M/55	HIV-1	Dementia, cough, night sweats, weight loss	0.5	7
5/M/32	*Aspergillus oryzae*	Headache, dizziness, diplopia, unilateral facial weakness and numbness	7	1
6/F/10	*Histoplasma capsulatum*	Back pain followed by unilateral facial droop, headache, neck stiffness	6	3.5
7/F/26	*Candida dubliniensis*	Low back pain followed by saddle anesthesia and unilateral foot drop	20	1

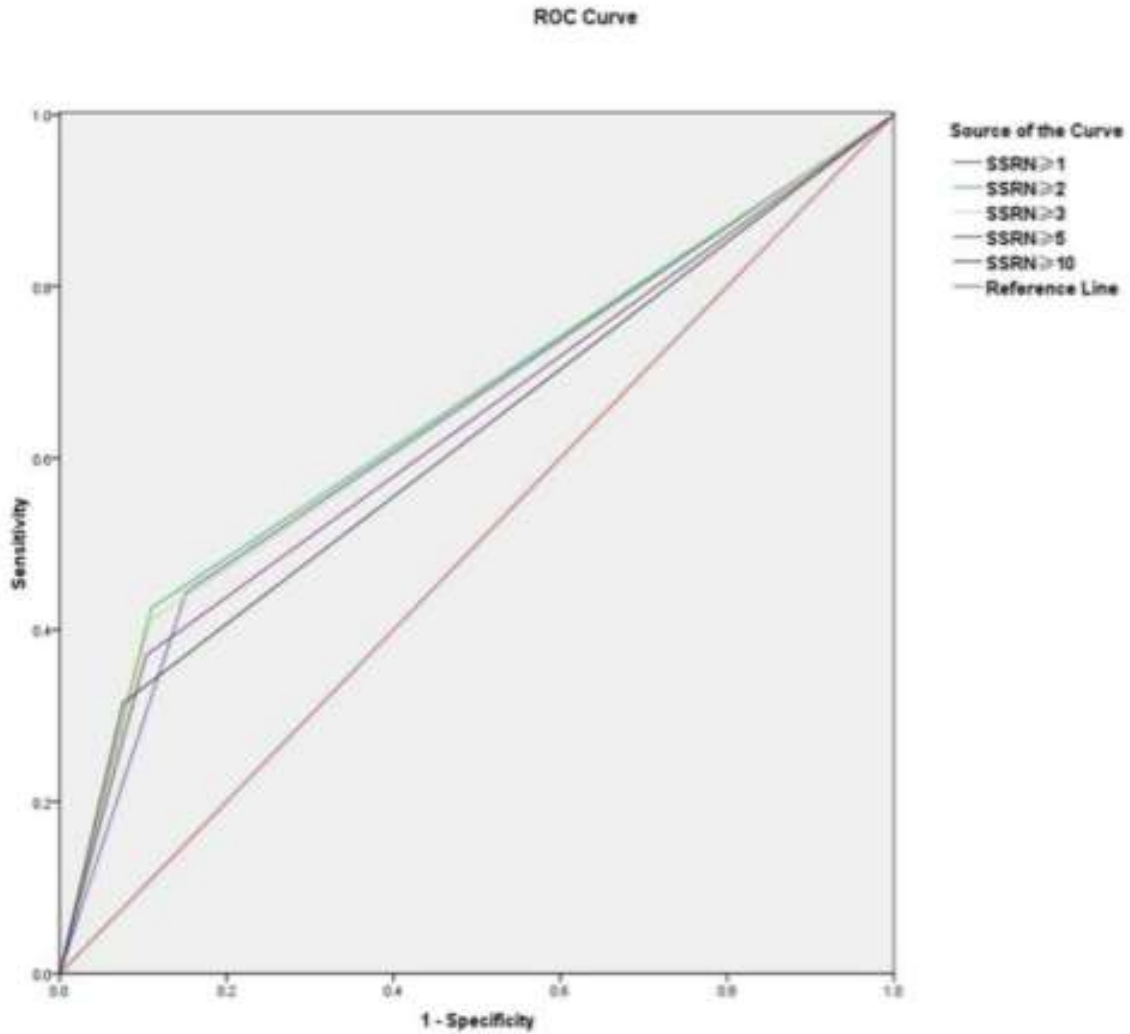
Metagenomic Next-Generation Sequencing for Diagnosis of Infectious Encephalitis and Meningitis: A Large, Prospective Case Series of 213 Patients

Xiao-Wei Xing[1], Jia-Tang Zhang[2,3], Yu-Bao Ma[2], Mian-Wa*
Wei Wang[5], Xiao-Kun Qi[6], Xiao-Yan Chen[2], Lei Wu[2], Xiao-
Juan Du[8], Hong-Fen Wang[2], Rong-Fei Wang[2], Fei Yang[2] a



Pathogen	SSRN	Coverage, %	Depth	
Viral encephalitis and/or meningitis (*n* = 23), SSRN ≥ 2				
Herpes simplex virus 1 (*n* = 9)	2,764 (2–27639)	57.9066 (0.0412–91)	2.99 (1–7.5)	PCR (*n* = 5), Positive HSV antibody (CLIA) (*n* = 9)
Herpes simplex virus 2 (*n* = 1)	4,311	90	6.5	Positive HSV antibody (CLIA)
Varicella-zoster virus (*n* = 5)	77 (6–3626)	4.3204 (0.2258–95.6227)	1 (1–2.47)	Skin herpes zoster (*n* = 4), PCR (*n* = 1)
Epstein-Barr virus (*n* = 6)	124.5 (4–3807)	6.8258 (0.1022–71.6965)	1 (1–2.39)	PCR (*n* = 3); Neuropathology (*n* = 1, DLBCL); Positive EBV antibody (*n* = 4)
Cytomegalovirus (*n* = 1)	4	0.081	1	CSF cytomegalovirus IgG (122.0 U/mL)
Human adenovirus B1 (*n* = 1)	2,467	62.309	9.84	Clinical evidence
Tuberculous meningitis (*n* = 12), GSRN ≥ 1				
Mycobacterium tuberculosis complex	4 (1–1046)	ND	ND	Xpert MTB/RIF (*n* = 3); A. TB or T-SPOT.TB (*n* = 4); Tuberculosis antibody (*n* = 1); Clinical evidence (*n* = 4)
Bacterial meningitis (*n* = 24), SSRN ≥ 5 or 10				
Streptococcus pneumoniae (*n* = 10)	2,488 (19–34711)	6.0112 (0.2236–66.8601)	1.31 (1–4.56)	Smear/culture (*n* = 4),
Streptococcus pyogenes (*n* = 1)	453	3.7	1	Smear
Streptococcus intermedius (*n* = 1)	592	5.2651	1	Clinical evidence
Klebsiella pneumoniae (*n* = 3)	15 (12–70)	0.0241 (0.0127–0.2979)	1 (1–1.59)	Culture (*n* = 3)
Listeria monocytogenes (*n* = 2)	43.5 (36–51)	0.1167 (0.0418–0.1915)	1.01 (1–1.02)	Culture (*n* = 2)
Nocardia farcinica (*n* = 1)	277	0.2631	1.16	Clinical evidence
Brucella (*n* = 1)	18 (GSRN)	ND	ND	RBPT(+) and SAT(+)
Stenotrophomonas maltophilia (*n* = 1)	288	0.7879	1	Clinical evidence
Haemophilus influenzae (*n* = 1)	12	0.1478	1	Clinical evidence
Escherichia coli (*n* = 1)	58	1.2399	1	Culture
Aggregatibacter aphrophilus (*n* = 1)	256	0.7625	4.61	Clinical evidence
Neisseria meningitidis (*n* = 1)	4543	44.2621	1.82	Clinical evidence
Fungal meningitis (*n* = 14), SSRN or GSRN≥2				
C. neoformans s.l. (*n* = 10)	40.5 (2–177203)	0.01445 (0.0019–71)	1 (1–24)	India ink staining or culture for fungi
C. gattii s.l. (*n* = 5)	334 (7–71743)	0.1885 (0.0136–20)	1.02 (1–8.7)	India ink staining or culture for fungi
Aspergillus (*n* = 4)	6 (3–9) (GSRN)	ND	ND	Histopathology

A


ROC Curve
Sensitivity
1 - Specificity
Source of the Curve
SSRN≥1
SSRN≥2
SSRN≥3
SSRN≥5
SSRN≥10
Reference Line

B

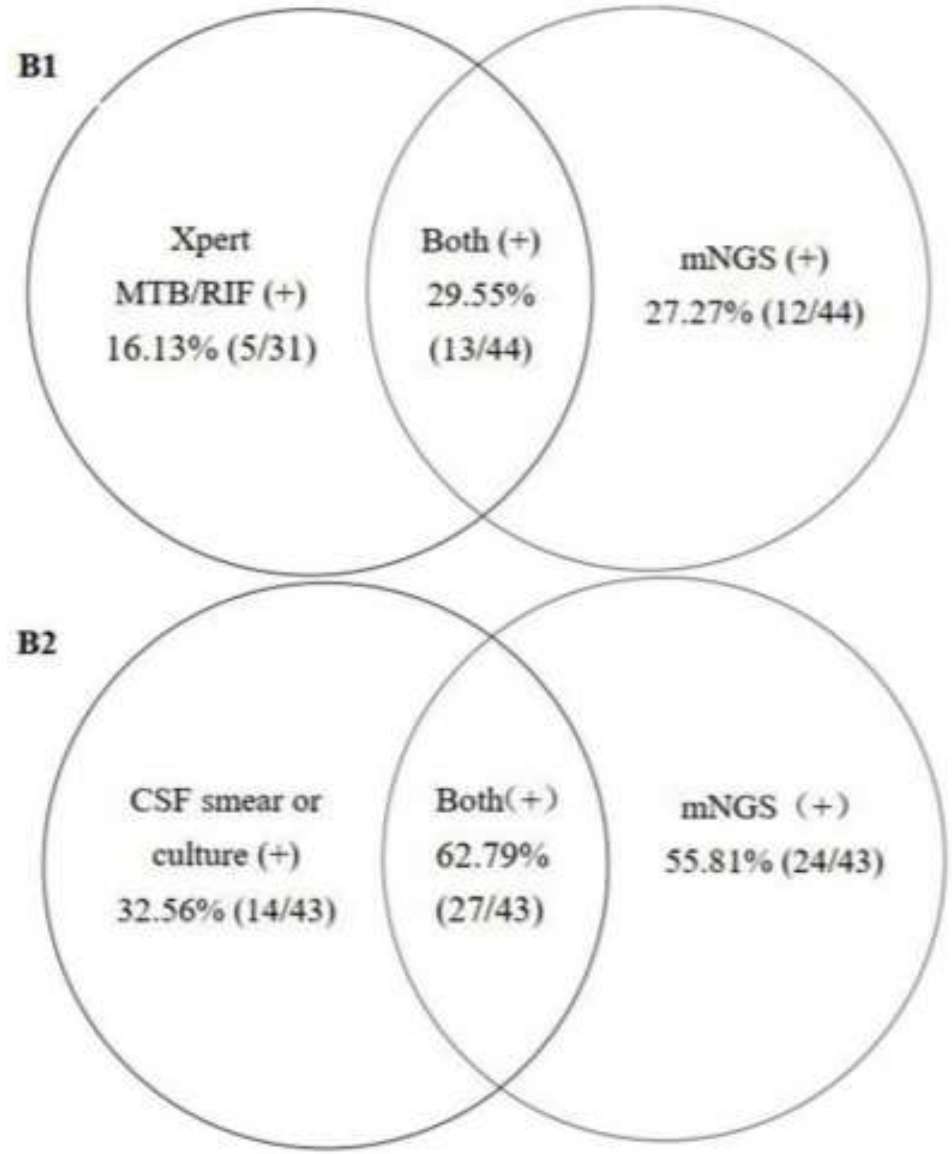
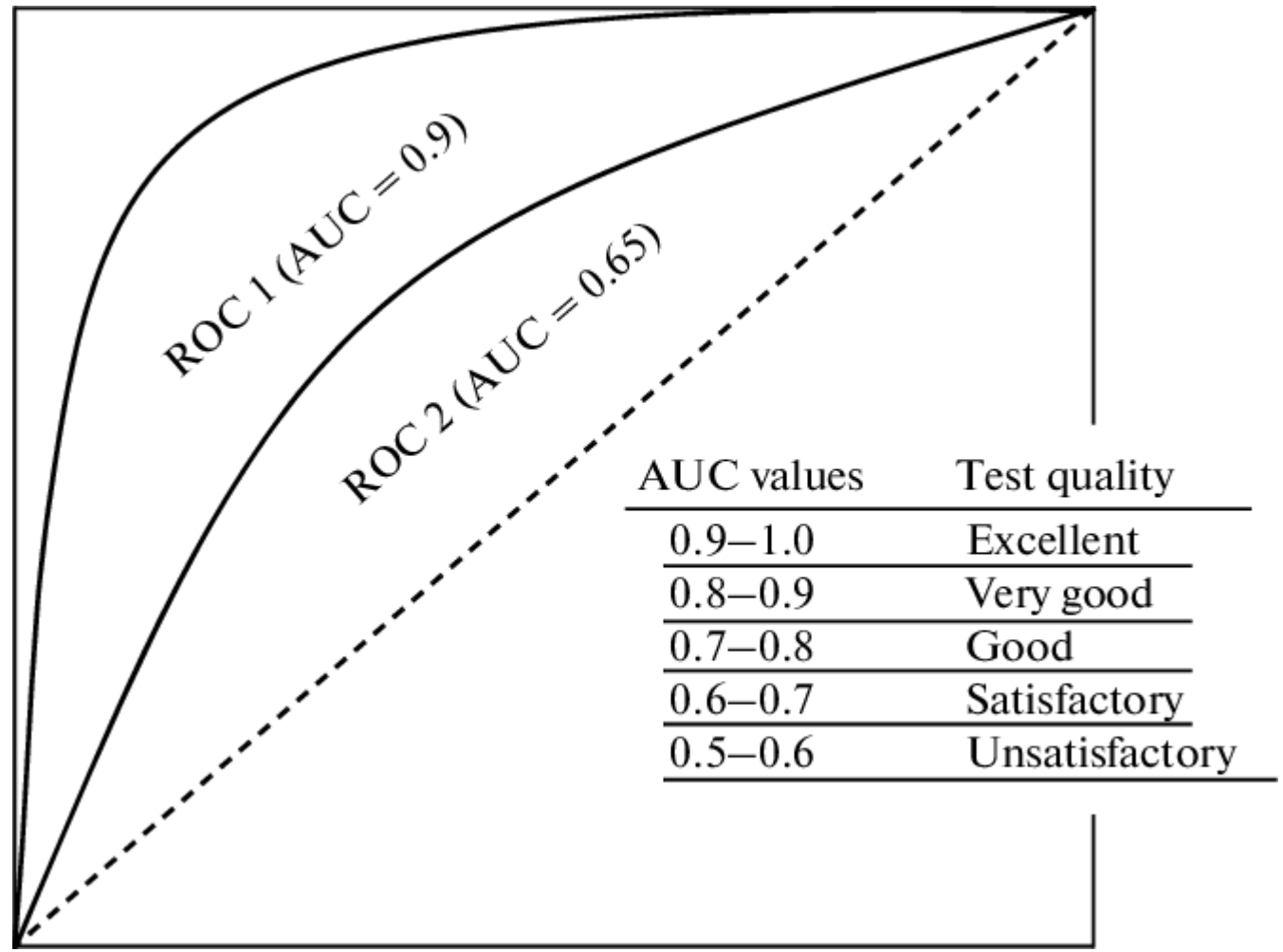

B1
Xpert
MTB/RIF (+)
16.13% (5/31)
Both (+)
29.55%
(13/44)
mNGS (+)
27.27% (12/44)
B2
CSF smear or
culture (+)
32.56% (14/43)
Both(+)
62.79%
(27/43)
mNGS (+)
55.81% (24/43)

TABLE 2 | Comparison of the diagnostic efficacy of five positive mNGS criteria for viral encephalitis and/or meningitis (Definite, $n = 54$).

SSRN	Positive consistent rate	Negative consistent rate	Total consistent rate	AUC	SE	95% CI
≥1	0.444	0.848	0.734	0.646	0.047	0.554–0.738
≥2	0.426	0.891	0.760	0.659	0.047	0.566–0.751
≥3	0.407	0.899	0.760	0.653	0.047	0.560–0.746
≥5	0.370	0.899	0.760	0.634	0.048	0.541–0.728
≥10	0.315	0.928	0.755	0.621	0.048	0.527–0.715

SSRN, species-specific read number; AUC, area under the curve; SE, standard error; CI, confidence interval.

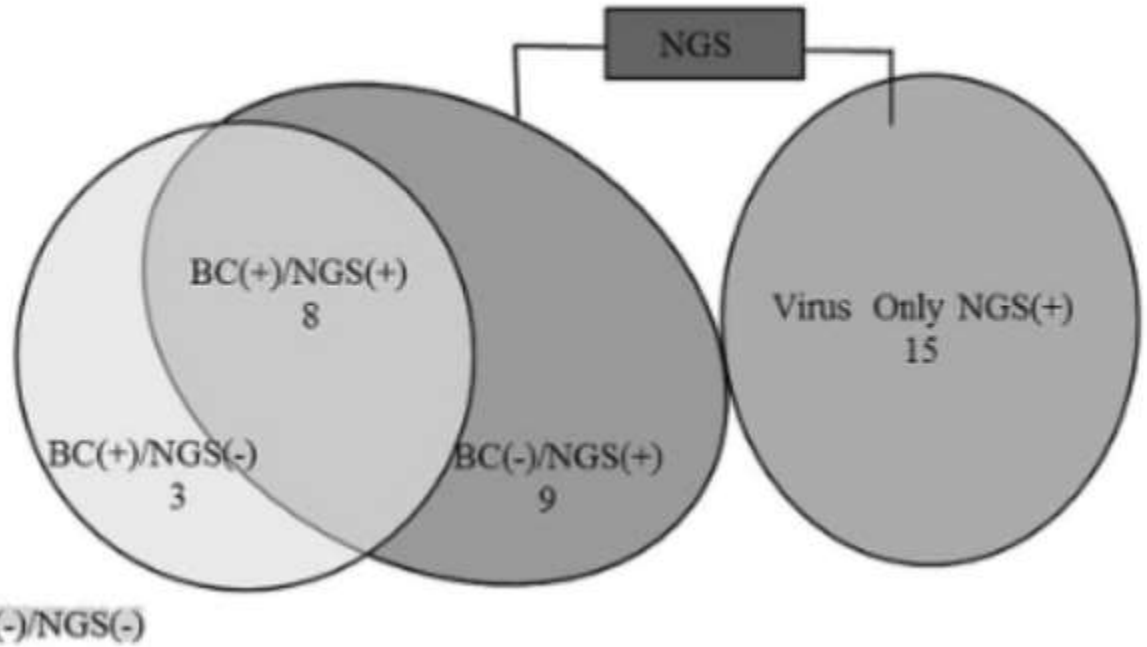


AUC values	Test quality
0.9–1.0	Excellent
0.8–0.9	Very good
0.7–0.8	Good
0.6–0.7	Satisfactory
0.5–0.6	Unsatisfactory

ORIGINAL ARTICLE

Diagnosis of Sepsis with Cell-free DNA by Next-Generation Sequencing Technology in ICU Patients

Yun Long,[a,*] Yinxin Zhang,[b,d,e,*] Yanping Gong,[b,d,e,*] Ruixue Sun,[b,d,e,*] Longxiang Su,[a] Xin Lin,[b,d,e]



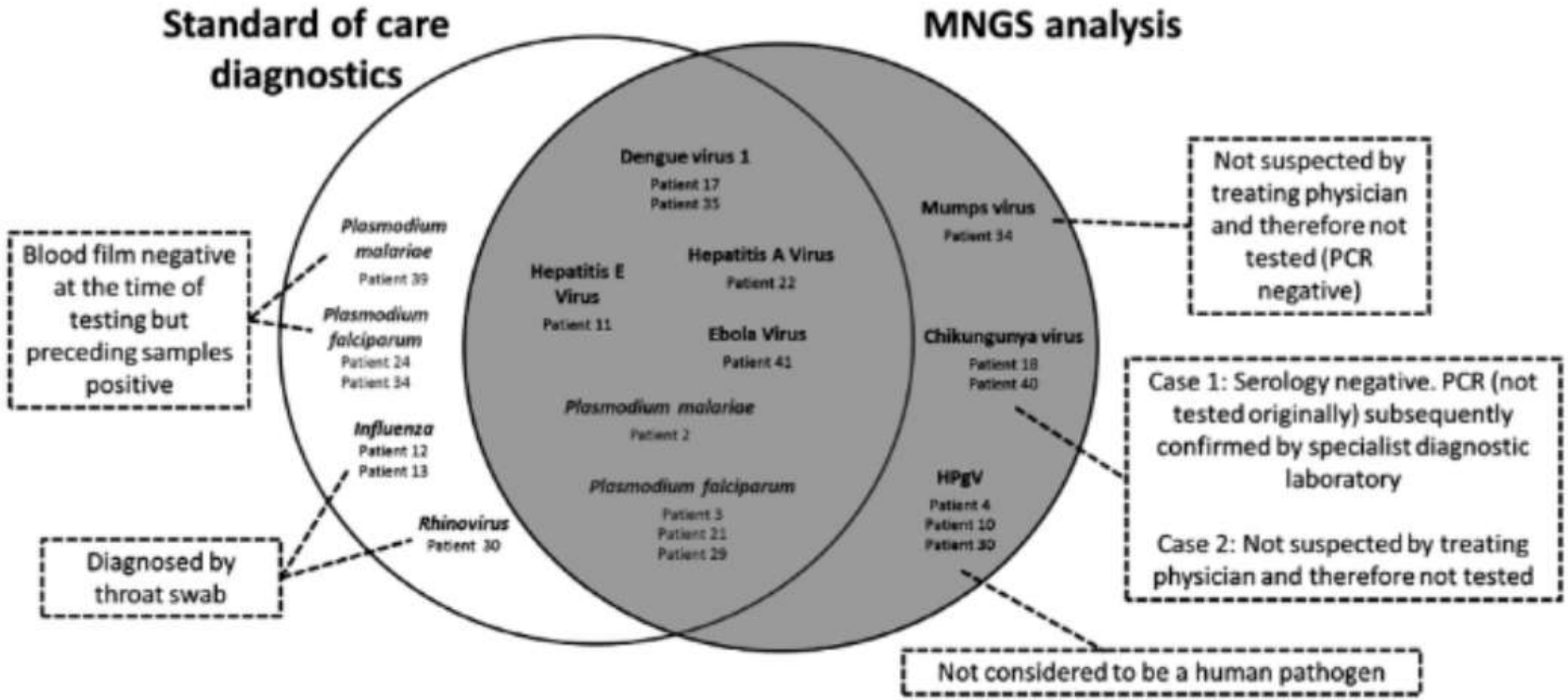
equality of proportions ($p = 0.013$). In addition, 15 viral pathogens including CMV, EBV, HSV-1 and human parvovirus B19 were identified in 14 samples by NGS. Combined

analysis

Type	Pathogen	Strain identified		
		BC (+)	NGS (+)	BC and NGS
Gram-positive bacteria	*Enterococcus faecalis*	2	1	2
	Enterococcus faecium	1	3	3
	Lactococcus lactis	0	1	1
	Staphylococcus aureus	1	2	2
Gram-negative bacteria	*Acinetobacter baumannii*	2	1	2
	Aeromonas hydrophila	0	1	1
	Bacteroides fragilis	1	1	1
	Citrobacter freundii	1	1	1
	Escherichia coli	0	1	1
	Klebsiella pneumoniae	1	3	3
	Pseudomonas aeruginosa	1	2	2
Fungi	*Candida albicans*	1	0	1
	Total	11	17	20

Metagenomic next-generation sequencing aids the diagnosis of viral infections in febrile returning travellers

Hanna Jerome[a,1], Callum Taylor[b,1], Vattipally B. Sreenu[a], Tanya Klymenko[a],



Methods: Plasma samples from 40 returning travellers presenting with a fever of ≥38°C were sequenced using MNGS on the Illumina MiSeq platform and compared with standard-of-care diagnostic assays.
Results: In total, 11/40 patients were diagnosed with a viral infection. Standard of care diagnostics re-

Retrospective clinical and microbiologic analysis of metagenomic next-generation sequencing in the microbiological diagnosis of cutaneous infectious granulomas

Hsingmei Liu[1†], Qiao Ran[1,2†], Jianchi Ma[1], Jing Zhang[1], Ni Tan[1,3], Liyan Xi[1], Xiqing Li[1], Junmin Zhang[1] and Sha Lu[1*]

Clinical and diagnostic values of metagenomic next-generation sequencing for infection in hematology patients: a systematic review and meta-analysis

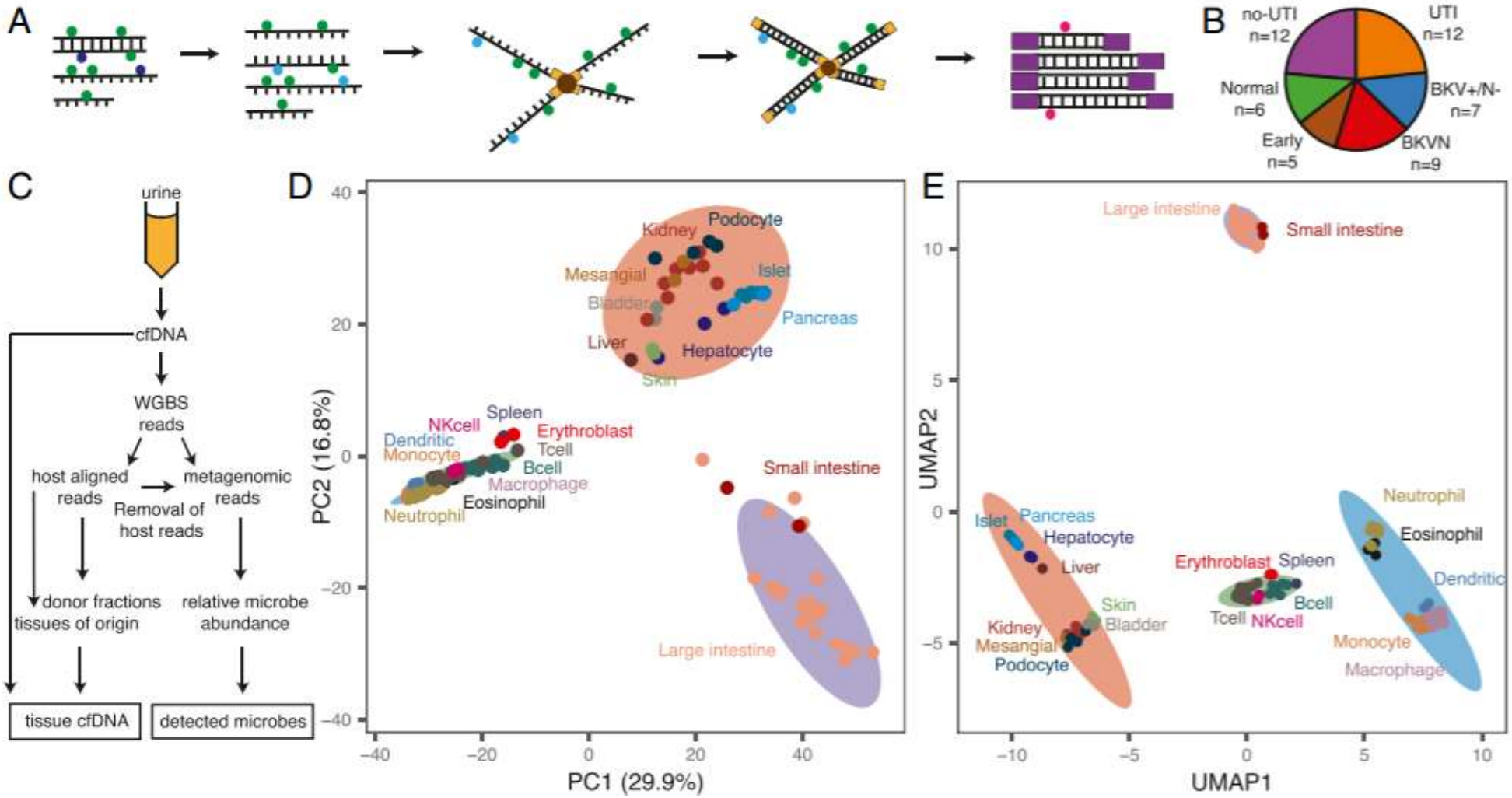
Yuhui Chen[1†], Jinjin Wang[1†] and Ting Niu[1*]

Conclusion mNGS has high positive detection rates in hematology patients. mNGS can guide clinical antibiotic adjustments and improve prognosis, especially in China.

Conclusions mNGS could provide a potentially rapid and effective alternative detection method for diagnosis of cutaneous infectious granulomas and mNGS results may affect the clinical prognosis resulting from enabling the patients to initiate timely treatment.

A cell-free DNA metagenomic sequencing assay that integrates the host injury response to infection

Alexandre Pellan Cheng[a], Philip Burnham[a], John Richard Lee[b,c], Matthew Pellan Cheng[d,e], Manikkam Suthanthiran[b,c], Darshana Dadhania[b,c,1,2], and Iwijn De Vlaminck[a,1,2]



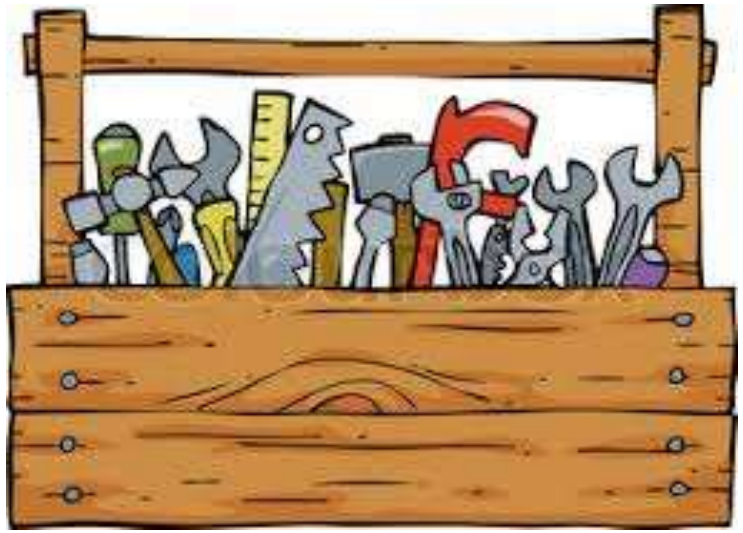
The challenge of diagnostic metagenomics

Alexander L. Greninger

Başlangıç : 410.000 $

Yıllık: 435.000 $ - 475.000 $

*Test Başı Maliyet; 2.000-2500 $



Teşekkürler…