

İnfeksiyon Hastalıklarında Yeni Moleküler Tanı Yöntemleri

Merkezi Sinir Sistemi İnfeksiyonları Paneli

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Anabilim Dalı

Merkezi Sinir Sistemi İnfeksiyonları

- Bakteriyel, fungal, viral, paraziter > 100 etken
- Modern tanı tekniklerine rağmen hala olguların %70'lere varan oranında etioloji belirlenemiyor
- Zaman → Çoklu saptama

- Nükleik asit teknolojileri (NAT):
 - Hibridizasyona dayalı (amplifikasyonsuz):
Sıvı, katı, *insitu*

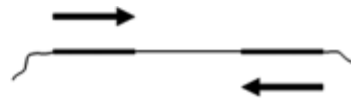
Amplifikasyona dayalı NAT

- **Sinyal amplifikasyonu:** bDNA, hibrid yakalama
- **Hedef amplifikasyonu:** PCR, TMA, SDA, NASBA, HDA, LAMP, MLPA
- **Prob amplifikasyonu:** LCR, cycling probe technology, Cleavase (-invader) technology
- **Mikroarray teknolojileri**
- **Yeni kuşak dizileme**

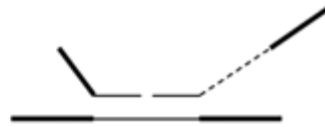
- Çoklu saptama tabanları:
 - Multipleks PCR
 - Real-time PCR
 - Boncuk tabanlı (luminex)
 - Kütle işaretli (Mass-Tag)
 - MLPA
 - Array teknolojileri
 -

MLPA (Multiplex ligation dependent probe amplification) - Meningofinder

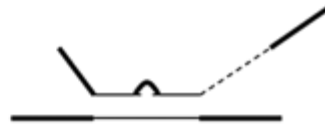
RT-PCR



Probe hybridization



Probe ligation



Probe amplification



Detection



En yaygın 6 virus:

enterovirus

cytomegalovirus (CMV)

varicella-zoster virus (VZV)

Epstein-Barr virus (EBV)

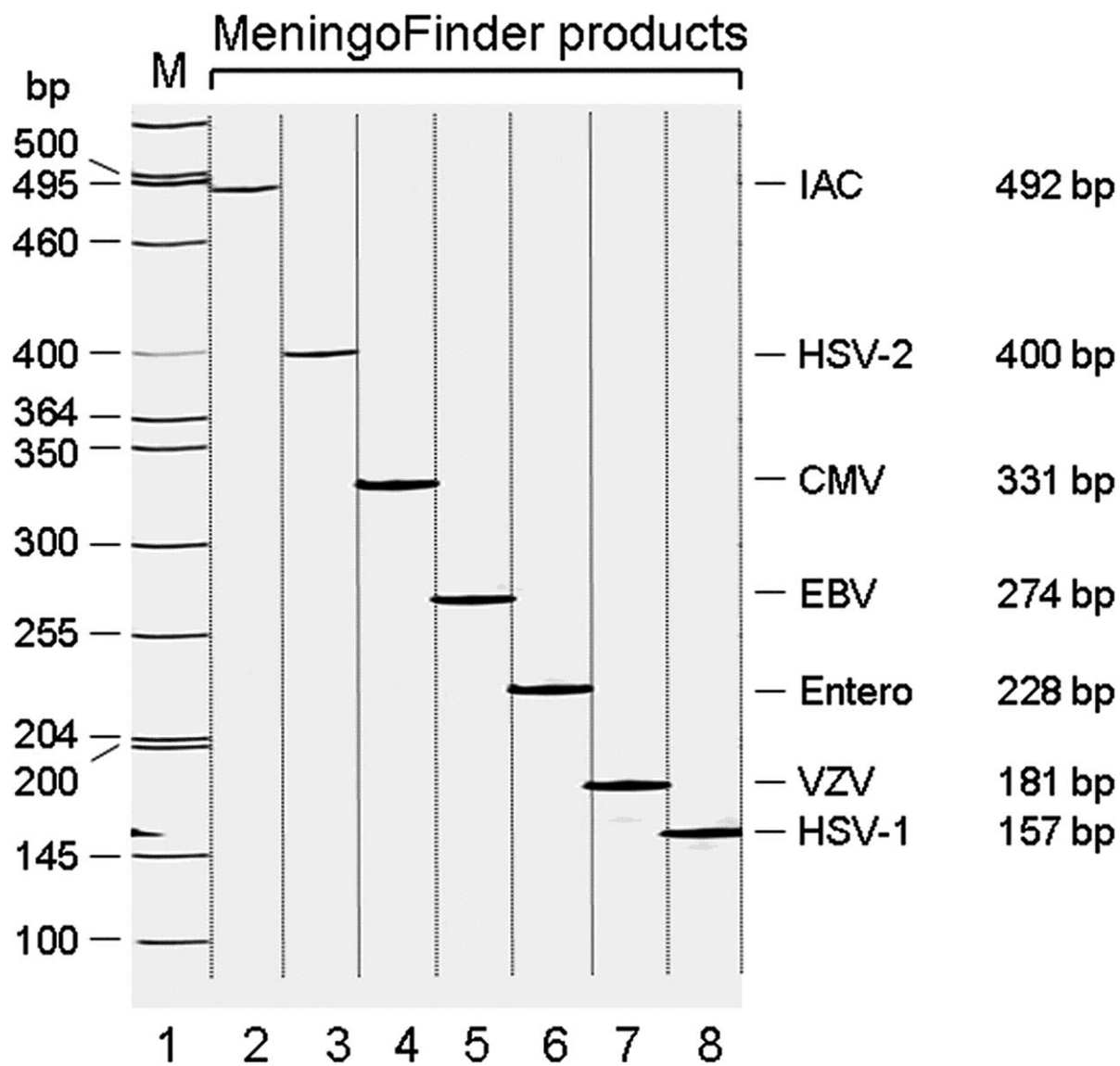
herpes simplex virus type 1
ve 2 (HSV-1 ve HSV-2).

- [J Clin Microbiol.](#) 2009 Aug;47(8):2620-2. doi: 10.1128/JCM.02436-08. Epub 2009 Jun 24.

Evaluation of MeningoFinder, a novel multiplex ligation-dependent probe amplification assay for simultaneous detection of six virus species causing central nervous system infections.

[Wolffs PF](#), [Vink C](#), [Keijdener J](#), [Habek B](#), [Reijans M](#), [Simons G](#),
[Bruggeman CA](#), [van den Brule AJ](#).

Department of Medical Microbiology, Maastricht University Medical Center, The Netherlands. p.wolffs@mumc.nl



Comparison of the results of MeningoFinder MLPA and PCR on 50 clinical CSF samples

No. of samples	PCR result ^a	MeningoFinder MLPA result by: ^b	
		PAMM	PF
12	Enterovirus	Enterovirus	Enterovirus
2	Enterovirus	Enterovirus ^c	Enterovirus
1	Enterovirus	Enterovirus ^c	HSV-2 + enterovirus
1	NEG	EBV	EBV
4	HSV-1	HSV-1	HSV-1
1	HSV-2	HSV-2	HSV-2
2	HSV-2 + EBV	HSV-2	HSV-2
4	VZV	VZV	VZV
1	NEG	VZV	NEG
22	NEG	NEG	NEG

- 4 kopya/MLPA assay - CMV
- 50 kopya/MLPA assay – EBV
- 15 kopya/MLPA assay- enterovirus
- 28 kopya/MLPA assay - HSV-1
- 180 kopya/MLPA assay - HSV-2
- 154 kopya/MLPA assay - VZV

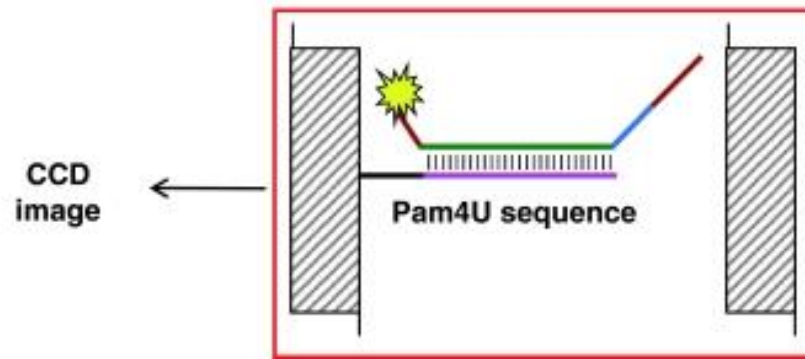
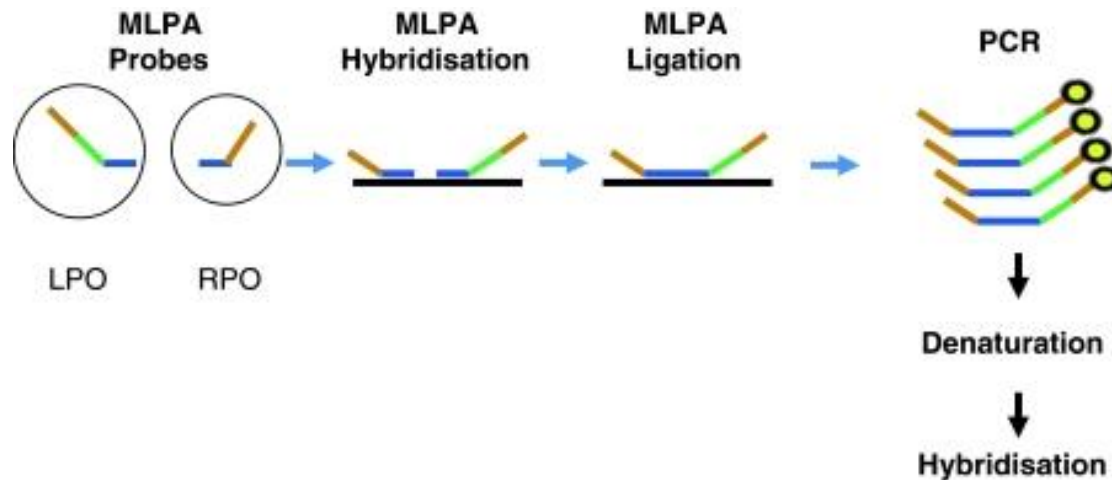
Mol Biotechnol. 2011 October; 49(2): 176–186.

Published online 2011 March 10. doi: [10.1007/s12033-011-9389-3](https://doi.org/10.1007/s12033-011-9389-3)

PMCID: PMC3172416

- **Development of a Flow-Trough Microarray based Reverse Transcriptase Multiplex Ligation-Dependent Probe Amplification Assay for the Detection of European Bunyaviruses**

[Lekbira Hasib](#), ¹ [Meik Dilcher](#), ¹ [Frank Hufert](#), ¹ [Ursula König-Meyer](#), ² and [Manfred Weidmann](#)



- TOSV
- SFSV TOSV, BATV, UUKV ve INKV → 10 kopya,
- SFNV
- TAHV SFSV, SFNV ve TAHV → 1000 kopya
- INKV
- BATV
- UUKV

Table 2

Analytical sensitivities of various assay types during the MLPA-microarray development given as molecules detected

Virus assay	RT-PCR	MLPA	MLPA-microarray
TOSV	10^1	10^1	10^1
SFSV	10^2	10^2	10^3
SFNV	10^2	10^2	10^3
TAHV	10^2	10^2	10^3
INKV	10^3	10^1	10^1
BATV	10^1	10^1	10^1
UUKV	10^1	10^1	10^1

Multiplex RT-PCR DNA microarray -Clart Entherpex kit

- HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8 ve HEV

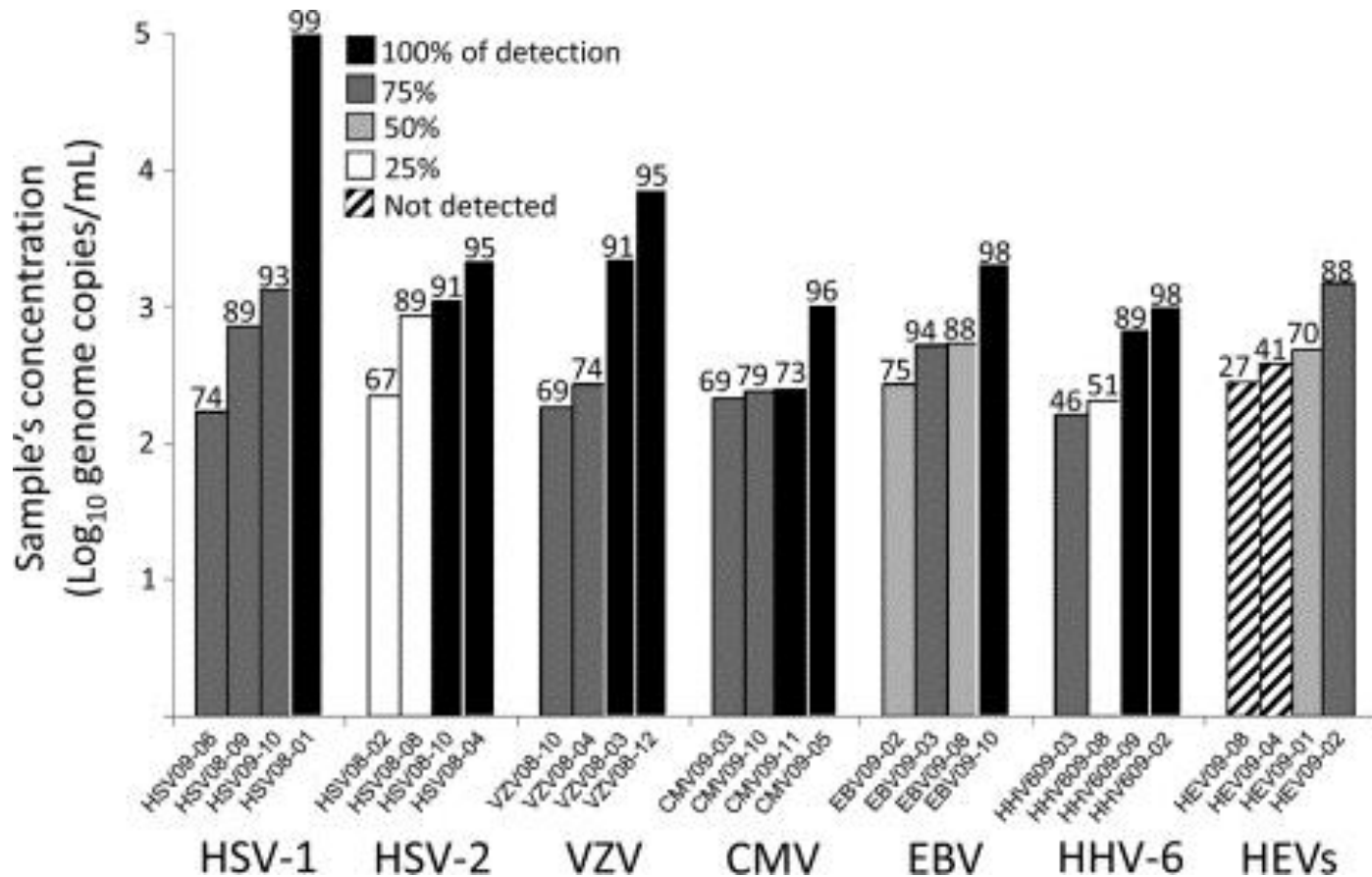
Multiplex PCR ve monoplex RT-PCR

- **Rapid Virological Diagnosis of Central Nervous System Infections by Use of a Multiplex Reverse Transcription-PCR DNA Microarray**

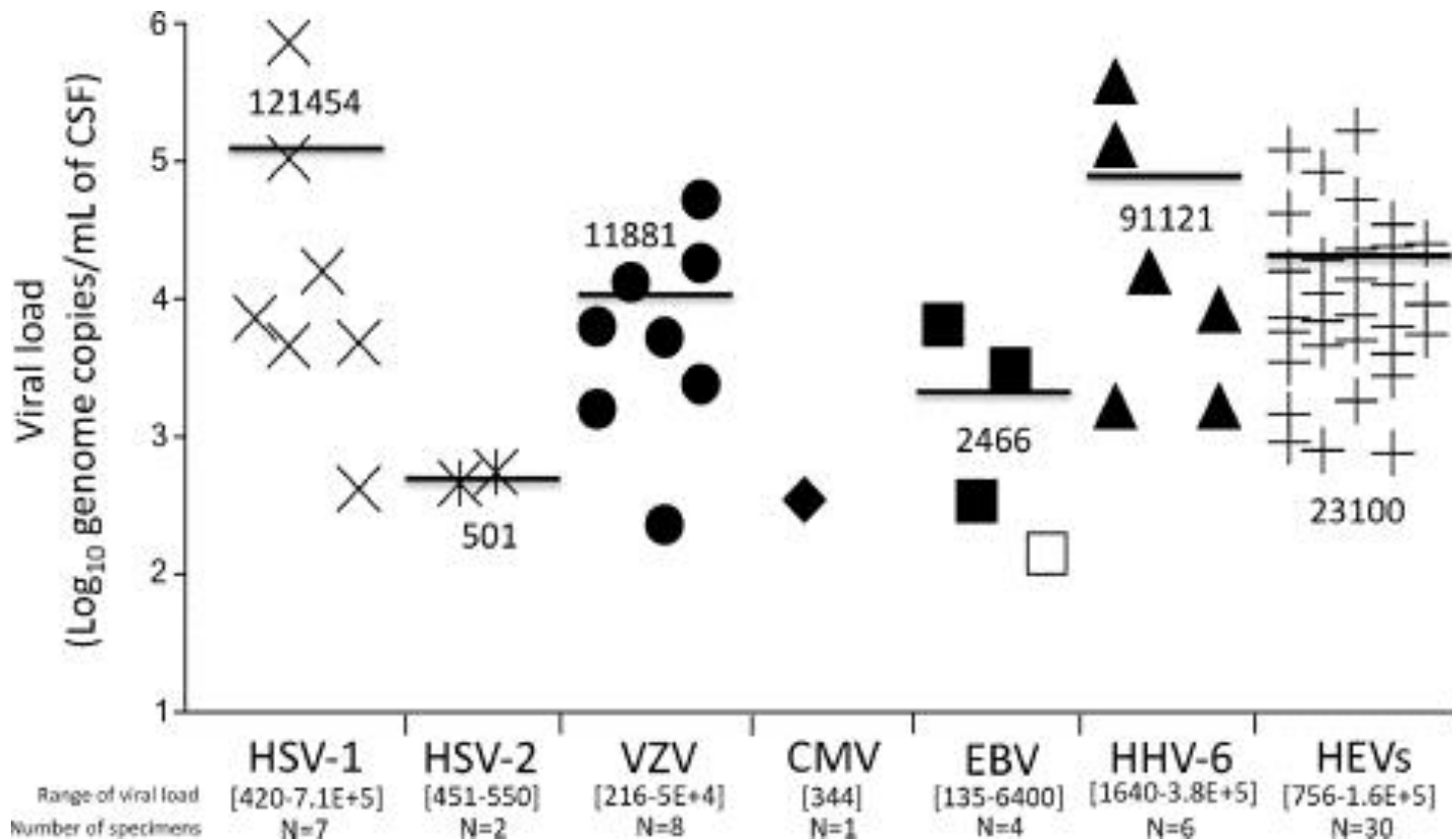
[Nicolas Leveque](#),^{1,*} [Adrien Van Haecke](#),¹ [Fanny Renois](#),¹ [David Boutolleau](#),² [Deborah Talmud](#),¹ and [Laurent Andreoletti](#)¹

J Clin Microbiol. 2011 November; 49(11): 3874–3879

4 QCMD panel



58'i pozitif 78 BOS örneği



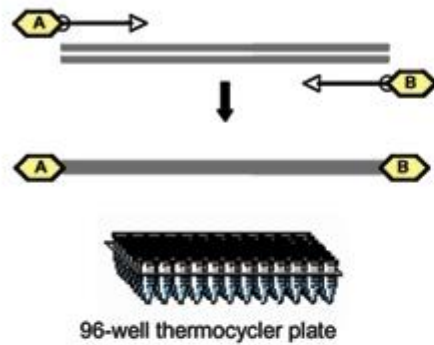
- Altın standart kabul edilen PCR (Argene) ile karşılaştırıldığında duyarlılığı % 98.3, özgüllüğü % 100, PPD % 100, 9NPD % 95.2
- Mikroarray HEV pozitif 30 çocuk hastanın 11'inde eşzamanlı olarak HHV-7 de saptıyor.

Emerg Infect Dis. 2005 February; 11(2): 310–313.

Diagnostic System for Rapid and Sensitive Differential Detection of Pathogens

[Thomas Briese](#),*,¹ [Gustavo Palacios](#),*,¹ [Mark Kokoris](#),†,¹ [Omar Jabado](#),* [Zhiqiang Liu](#),* [Neil Renwick](#),* [Vishal Kapoor](#),* [Inmaculada Casas](#),‡ [Francisco Pozo](#),‡ [Ron Limberger](#),§ [Pilar Perez-Brena](#),‡ [Jingyue Ju](#),* and [W. Ian Lipkin](#) *

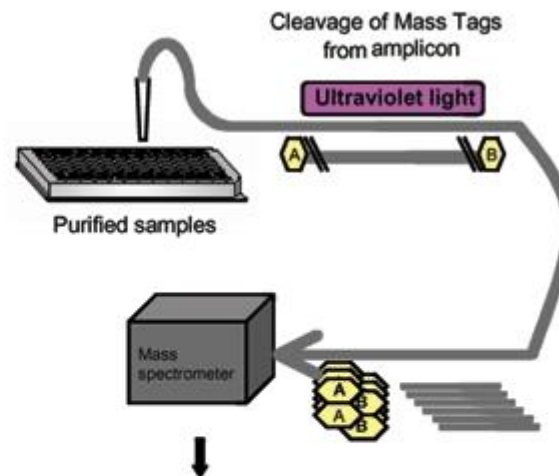
1. PCR amplification with Mass Tag primers 4. Automated sample injection, photocleavage



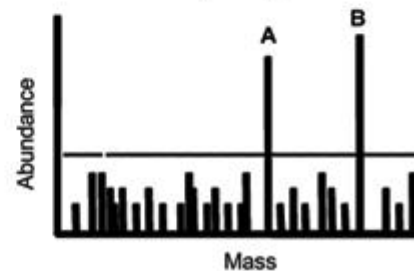
2. Product purification on filter plate



3. Elution into 96-well loading plate for mass spectrometer analysis



5. Detection and pathogen identification



- 17- pleks → Bakteri + Virus
- İnfluenza A virus; influenza B virus; human coronaviruses 229E, OC43 ve SARS; enterovirus; adenovirus; human herpesvirus-1 ve -3; West Nile virus; St. Louis encephalitis virus; measles virus; HIV-1 ve -2;
- *Streptococcus pneumoniae*, *Haemophilus influenzae* ve *Nisseria meningitidis*

2011-2013 arası 179 BOS örneğinde

HSV TIP I	7
EBV	5
VZV	2
HSVII	0

Evaluation of the Seeplex® Meningitis ACE Detection Kit for the Detection of 12 Common Bacterial and Viral Pathogens of Acute Meningitis

So Youn Shin, M.D.,^{1,2} Kye Chul Kwon, M.D.,¹ Jong Woo Park, M.D.,¹ Ji Myung Kim, M.D.,¹ So Young Shin, M.D.,³ and Sun Hoe Koo, M.D.¹ Dual priming oligonucleotide (DPO) yöntemi ile Multiplex PCR paneli

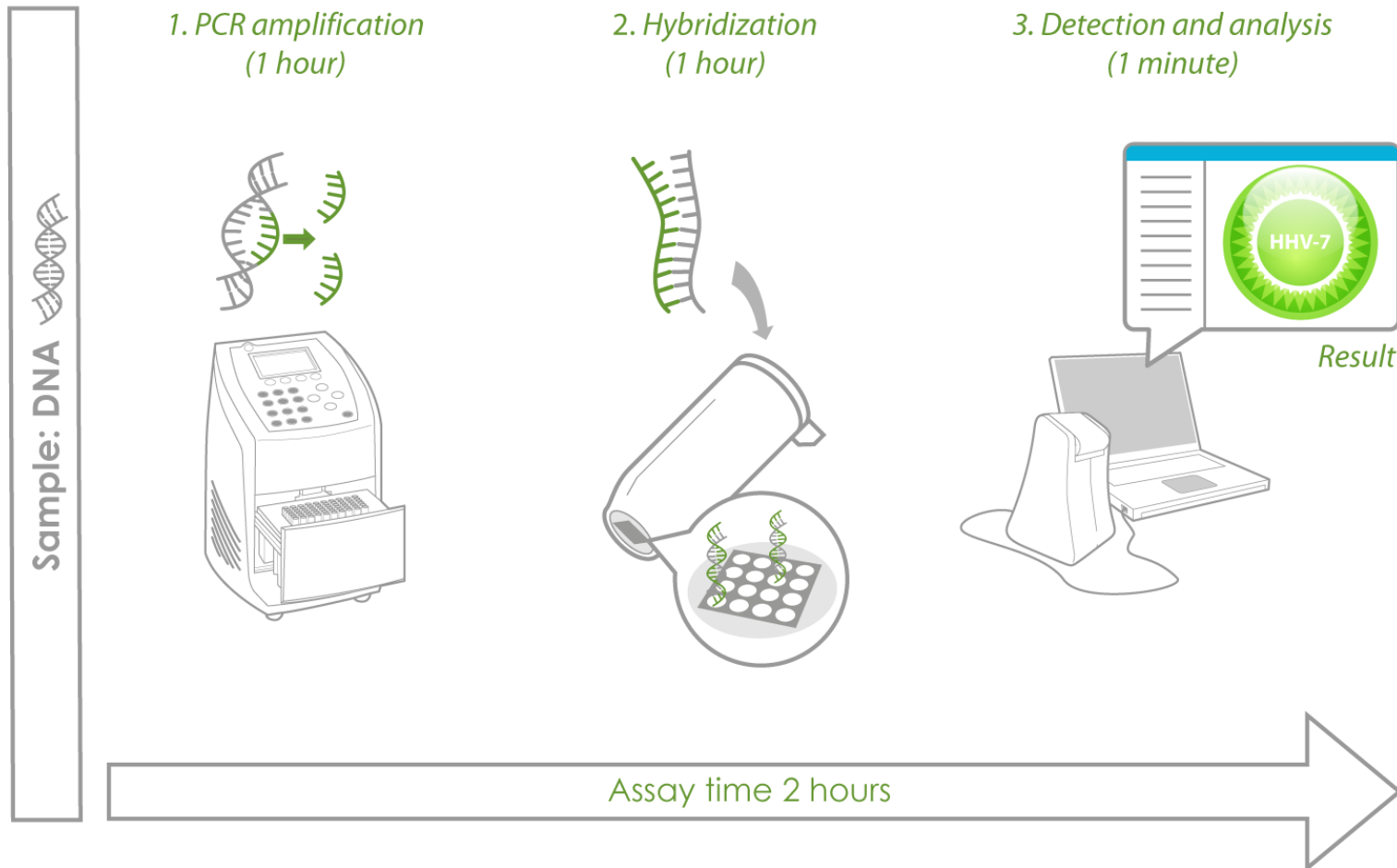
- En sık rastlanan 12 viral ve bakteriyel patojen:
- *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, Group B streptococci, *Listeria monocytogenes*,
- HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HEV.
- Saptama limiti 10-50 kopya (Üretici 100 kopya ve üstü)
- 78 BOS örneği ve 27 kontrol BOS'u incelenmiş, Çapraz reaksiyon ve yanlış pozitif sonuç yok.

Fast Track Diagnostik

- Viral etkenler için
- HSV-1, HSV-2 VZV, Enterovirus ve Kabakulak virusunu saptayabilen iki tüplü multipleks, realtime-PCR tabanlı kiti mevcut,
- Bakteriyel etkenler için *S. pneumoniae*, *N. meningitidis* ve *H. influenzae*'yi saptayabilen tek tüp içinde multipleks, realtime-PCR tabanlı kiti mevcut
- Saptama limiti 10-100 kopya

Tüp Array

HSV-2, EBV, VZV, CMV, HHV6, HHV7



Eight-Plex PCR and Liquid-Array Detection of Bacterial and Viral Pathogens in Cerebrospinal Fluid from Patients with Suspected Meningitis[∇]

Mette Kusk Bøving, Lisbeth Nørum Pedersen and Jens Kjølseth Møller*

- Luminex tabanlı çoklu saptama s,stem,
- Neisseria meningitidis, Streptococcus pneumoniae, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Streptococcus agalactiae
- HSV-1, HSV-2 VZV.
- 1,187 örnek ile çalışılıyor.
- Duyarlılık ve özgüllük
- S. pneumoniae için % 95 ve % 99.1,
- N. meningitidis için % 100 ve % 99.7

TABLE 3. Distribution of viral test results

Virus	Result ^a		Total no. of patient samples with a positive result
	Real-time PCR (gold standard)	PCR-Luminex assay	
VZV	+	+	6
	-	+	3
No. of samples positive	6	9	9
HSV	+	+	6
	-	+	1
No. of samples positive	6	7	7

^a + and -, samples positive or negative by the analysis applied.

**Astrovirus Encephalitis in Boy with
X-linked Agammaglobulinemia**

**Phenix-Lan Quan, Thor A. Wagner, Thomas Briese, Troy R. Torgerson, Mady Hornig,
Alla Tashmukhamedova, Cadhla Firth, Gustavo Palacios, Ada Baisre-De-Leon,
Christopher D. Paddock, Stephen K. Hutchison, Michael Egholm, Sherif R. Zaki,
James E. Goldman, Hans D. Ochs, and W. Ian Lipkin**

- Yeni Kuşak dizileme ile X'e bağlı agamaglobulinemisili 15 yaşında bir erkek hastada gelişen fatal seyirli ensefalitte astrovirus tanımlanıyor.
- 2008'de bir grup organ alıcısı hastada fatal seyreden ensefalitlerden yeni bir arenavirusun sorumlu olduğu gösterildi.

Accuracy of real-time PCR, Gram stain and culture for *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* meningitis diagnosis.

[Wu HM](#), [Cordeiro SM](#), [Harcourt BH](#), [Carvalho M](#), [Azevedo J](#), [Oliveira TQ](#), [Leite MC](#), [Salgado K](#), [Reis MG](#), [Plikaytis BD](#), [Clark TA](#), [Mayer LW](#), [Ko AI](#), [Martin SW](#), [Reis JN](#).

Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, CDC, Atlanta, GA 30333, USA. hmwu@emory.edu

- 451 BOS örneği, 80 (%17.7) kültür pozitif → 40 *S. pneumoniae*, 36 *N. meningitidis* ve 4 *H. Influenzae*
- RT ile 113 (%25.1%) pozitif → 51 *S. pneumoniae*, 57 *N. meningitidis* ve 5 *H. influenzae* Compared to culture, Kültür ile karşılaştırıldığında real-time PCR'in duyarlılık ve özgüllüğü % 95.0 ve % 90.0
- Latent class analysis modelinde duyarlılık ve özgülük tahminleri:
- Kültür için %81.3 ve %99.7
- Gram boyama için %98.2 ve %98.7
- Real-time PCR için %95.7% ve %94.3

Rapid detection of eight causative pathogens for the diagnosis of bacterial meningitis by real-time PCR.

[Chiba N](#), [Murayama SY](#), [Morozumi M](#), [Nakayama E](#), [Okada T](#), [Iwata S](#), [Sunakawa K](#), [Ubukata K](#).

Source

Laboratory of Molecular Epidemiology for Infectious Agents, Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo, 108-8641, Japan.

- Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Neisseria meningitidis, Escherichia coli, Streptococcus agalactiae, Staphylococcus aureus ve Listeria monocytogenes,
- Molecular beacon prob kullanılarak real time PCR ve kültür karşılaştırılıyor.
- 150 örnek YD ve çocuk; 18 erişkin Moleküler inceleme toplam 1.5 h
- Alt saptama sınırı 5-28 kopya/tüps
- 37 kontrolde nonspesifik amplifikasyon yok
- Real time PCR ile örneklerin % 72.0'sinde; kültür ile ise %48.2'sinde pozitiflik saptanıyor

[PLoS One](#). 2012;7(12):e52001. doi: 10.1371/journal.pone.0052001. Epub 2012 Dec 21. **Comparison of real time IS6110-PCR, microscopy, and culture for diagnosis of tuberculous meningitis in a cohort of adult patients in Indonesia.**

[Chaidir L](#), [Ganiem AR](#), [Vander Zanden A](#), [Muhsinin S](#), [Kusumaningrum T](#), [Kusumadewi I](#), [van der Ven A](#), [Alisjahbana B](#), [Parwati I](#), [van Crevel R](#).

Health Research Unit, Faculty of Medicine, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia. lidya.upk.unpad@gmail.com

- 230 ardışık hasta ve 40 kontrol
- 207 tbc ya da olası tbc hastası; hastaların % 17.9'u HIV pozitif bu grupta IS6110-PCR pozitifliği %68; mikroskopi ile pozitiflik %11;
- Sıvı besiyerinde kültürünki %44.

[J Clin Microbiol.](#) 2011 May;49(5):1861-5. doi: 10.1128/JCM.00824-10. Epub 2011 Mar 16.

Loop-mediated isothermal amplification for rapid and reliable diagnosis of tuberculous meningitis.

[Nagdev KJ](#), [Kashyap RS](#), [Parida MM](#), [Kapgate RC](#), [Purohit HJ](#), [Taori GM](#), [Daginawala HF](#).

Biochemistry Research Laboratory, Central India Institute of Medical Sciences, Nagpur 440010, Maharashtra, India.

- Loop-mediated isothermal amplification assay (LAMP) ile PCR karşılaştırması
- LAMP'in duyarlılık ve özgüllüğü: % 88.23 ve % 80,
- Nested-PCR'in duyarlılık ve özgüllüğü: % 52.9 ve %90.

- Geline nokta yeterli mi?
- Hala olguların çoğunda etken saptanamıyor
- Kantitasyon çok kısıtlı