Sekcija za klinično mikrobiologijo in bolnišnične okužbe Slovenskega zdravniškega društva

in

Inštitut za mikrobiologijo in imunologijo Medicinske fakultete Univerze v Ljubljani

7. Likarjev simpozij – NOVI KONCEPTI V DIAGNOSTIČNI MIKROBIOLOGIJI

15. junij 2017 City Hotel Ljubljana, Dalmatinova 15

Mario Poljak - Pregled novosti na področju diagnostične mikrobiologije in kaj nas čaka v bližnji in daljni prihodnosti - stran 2

Katja Seme - Avtomatizacija bakteriološkega laboratorija - stran 52

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Pregled novosti na področju diagnostične mikrobiologije in kaj nas čaka v bližnji ter daljni prihodnosti

Mario Poljak

Inštitut za mikrobiologijo in imunologijo
Medicinska fakulteta, Univerza v Ljubljani

AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

EDITORIAL

Revolutionary Science

Arturo Casadevall,[a] Founding Editor in Chief, *mBio*, **Ferric C. Fang,**[b] Editor in Chief, *Infection and Immunity*

Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA[a]; Departments of Laboratory Medicine and Microbiology, University of Washington School of Medicine, Seattle, Washington, USA[b]

ABSTRACT **On rare occasions in the history of science, remarkable discoveries transform human society and forever alter mankind's view of the world. Examples of such discoveries include the heliocentric theory, Newtonian physics, the germ theory of disease, quantum theory, plate tectonics and the discovery that DNA carries genetic information. The science philosopher Thomas Kuhn famously described science as long periods of normality punctuated by times of crisis, when anomalous observations culminate in revolutionary changes that replace one paradigm with another. This essay examines several transformative discoveries in the light of Kuhn's formulation. We find that each scientific revolution is unique, with disparate origins that may include puzzle solving, serendipity, inspiration, or a convergence of disparate observations. The causes of revolutionary science are varied and lack an obvious common structure. Moreover, it can be difficult to draw a clear distinction between so-called normal and revolutionary science. Revolutionary discoveries often emerge from basic science and are critically dependent on non-revolutionary research. Revolutionary discoveries may be conceptual or technological in nature, lead to the creation of new fields, and have a lasting impact on many fields in addition to the field from which they emerge. In contrast to political revolutions, scientific revolutions do not necessarily require the destruction of the previous order. For humanity to continue to benefit from revolutionary discoveries, a broad palette of scientific inquiry with a particular emphasis on basic science should be supported.**

"dramatic or wide-reaching change"

"significant societal benefit"

Revolution vs. evolution in diagnostic microbiology?

- molecular diagnostic microbiology
- MALDI-TOF mass spectrometry
- total laboratory automation in bacteriology
- syndrome-specific testing
- point-of-care tests and 24/7 concept
- digital PCR
- next-generation sequencing
- next-generation antimicrobial susceptibility testing
- CRISPR-Cas – based diagnostic assays
- non-microorganism detection based diagnostic approaches

revolution ?

molecular diagnostic microbiology

Molecular methods

dramatically changed clinical microbiology

allowed discovery of several clinically important and previously unrecognized or uncultivable pathogens

reduced the dependency of laboratory on culture-based methods

became gold diagnostic standards for several microorganisms

(*C. trachomatis*, HSV encephalitis, enteroviral meningitis, CMV reactivation, hepatitis C,…)

standardisation

automation

miniaturisation

m2000 (Abbott)

m2000sp

m24sp

m2000rt

Cobas Ampliprep/TaqMan system (Roche)

QIAsymphony SP | QIAsymphony AS

Molecular diagnostic systems 2.0+

- fully automated sample-to-result fashion
- multiple tests performed concordantly
- sample number flexibility
- STAT test prioritization

- random access

Panther System (Hologic-Gen-Probe)

Cobas 6800/8800 (Roche)

Beckman Coulter Presents The New Veris Mdx Molecular Diagnostics System

by JAN SINNIGE *on* May 13, 2014 • 6:03 pm

Beckman Coulter has unveiled its new random access molecular diagnostics system, the VERIS MDx, at the European Conference on Clinincal Microbiology and Infectious Diseases (ECCMID) in Barcelona this week. The VERIS MDx system and VERIS CMV assay received CE mark earlier this year.

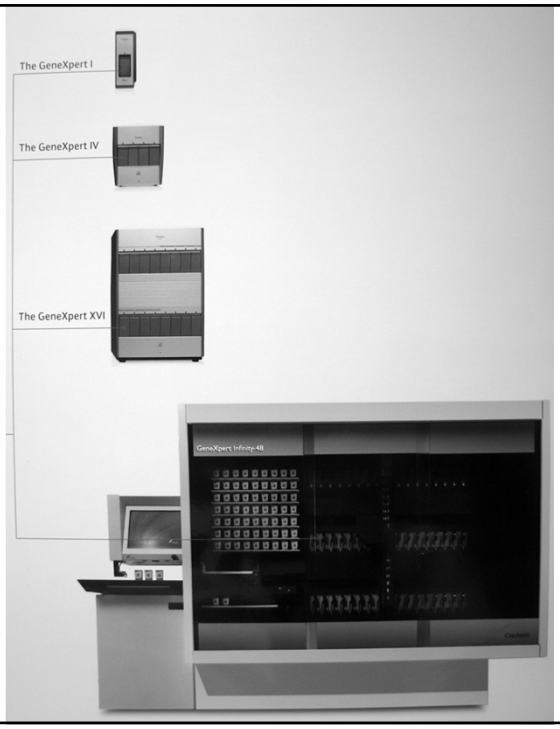
The system offers automated nucleic acid extraction, purification, amplification, and detection. It accepts several sample containers for plasma, serum and culture tubes. 48 samples can be lined up on 12 racks of 4 samples each. The time to result for DNA tests is around 70 minutes and for RNA tests a little longer, around 100 minutes, because PCR amplification only works on DNA and therefore you must reverse-transcribe to cDNA first. For multiplex analysis five different detection colors available with a bandwidth of 505 to 720 nm. The onboard capacity consists of 96 extraction and purification cartridges and reagents are covered for 20 assays with 48 tests per assay. Reagents are stable in the machine for up to 14 days.

Small/middle scale integrated systems

automated isolation of DNA or RNA
+
real-time PCR

GeneXpert (Cepheid)

single-use disposable cartridges

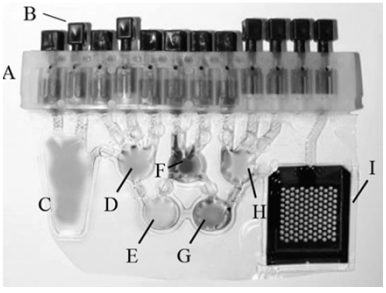


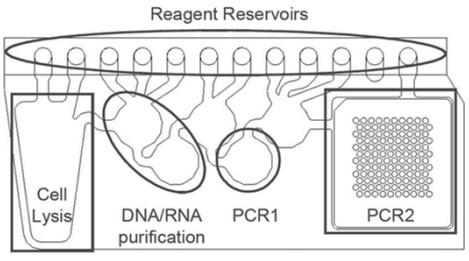
BD MAX System (Becton Dickinson)

Jaguar system (HandyLab)

3M Integrated Cycler (Focus)

FilmArray (Idaho Technology; BioFire Diagnostics; BioMerieux)





revolution ?

point-of-care tests and 24/7 concept

"3R" rule

Rapid	(in clinically relevant time frames)
Relevant	(clinically relevant)
Right	(specific and sensitive, analytical category)

~~Right > Relevant > Rapid~~

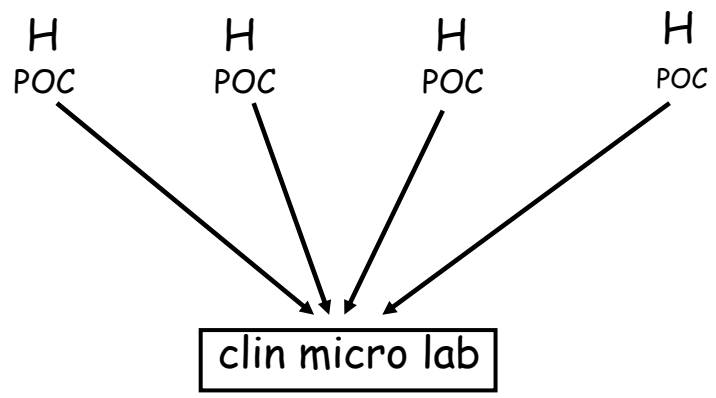
Relevant = Rapid > Right

faster

cheaper

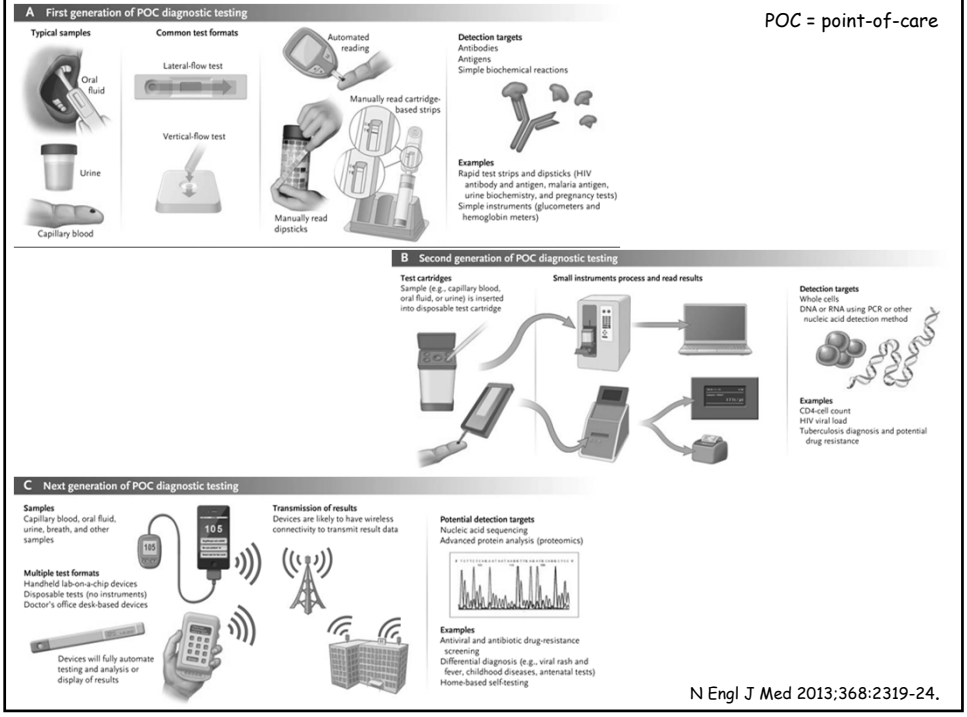
24/7

Two testing places – evolving concept



POC = point-of-care

POC = point-of-care



N Engl J Med 2013;368:2319-24.

Desire is to have self-contained, fully integrated sample-to-report devices that accept raw, untreated specimens, perform all of the molecular steps, and provide interpreted test results in < 1 h

Point-of-Care Molecular Testing

entering clinical practice throughout the world

paradigm shift towards decentralized testing

especially suited for applications:

- where fast turnaround is desirable
- where centralized laboratory services face limitations
- in resource-limited countries
- in rural areas and places that are hard to reach
- ships, submarines, off-shore platforms….(3D printer technology and remote fault diagnosis will allow reparation of failures using a small stock of materials and versatile components)

poses diverse technological, economic and organizational challenges

Fifteen-Minute Detection of *Streptococcus pyogenes* in Throat Swabs by Use of a Commercially Available Point-of-Care PCR Assay

James R. Uhl,[a] Robin Patel[a,b]

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA[a]; Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA[b]

J Clin Microbiol 2016;54:815

cobas Liat strep A assay vs. *S. pyogenes* LightCycler PCR assay

sensitivity = 100%

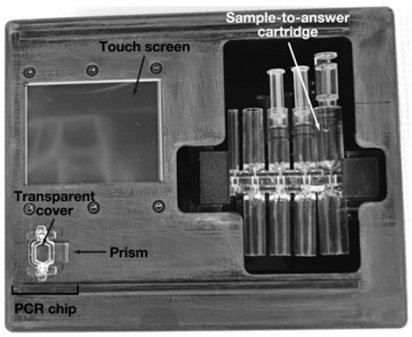
specificity = 98.3 %

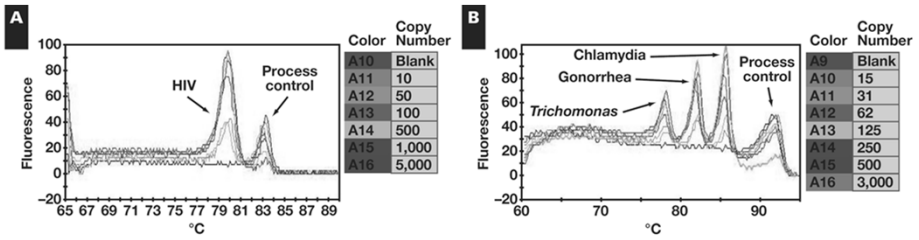
positive predictive value = 97.7%

negative predictive value = 100.0%

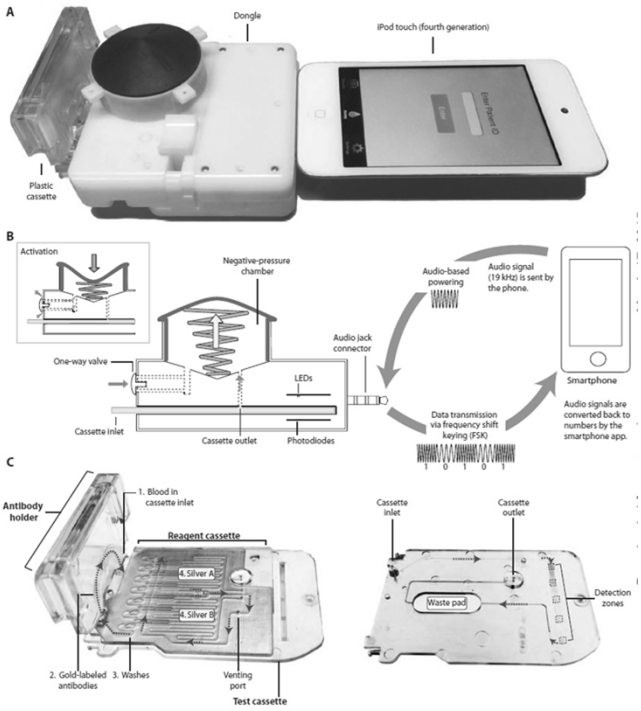
Where is my instrument ???

An iPad-like, sample-to-answer prototype





Abou Tayoun AN et al. Am J Clin Pathol 2014;141:17-24.



A smartphone dongle as point of care device

Sci Trans Med 2015; 7: 273re1

Lab-on-a-USB key

microfluidic devices integrated with USB key data storage devices

a device could be attached to other computational devices such as a cell phone or laptop computer to control molecular assays being done on the microfluidic biochip

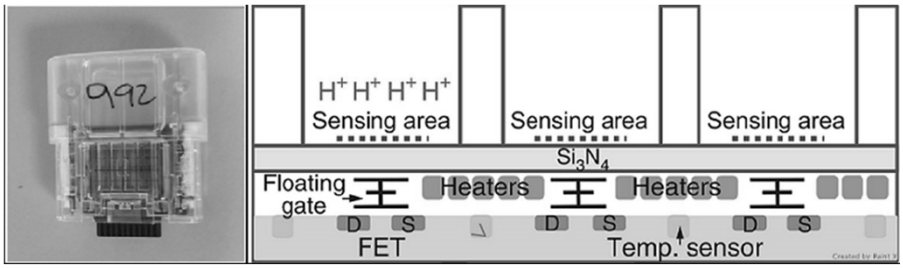
analysis transmitted to central databases for shared use and metaprocessing

Novel pH sensing semiconductor for point-of-care detection of HIV-1 viremia

Sci Rep 2016;6:36000

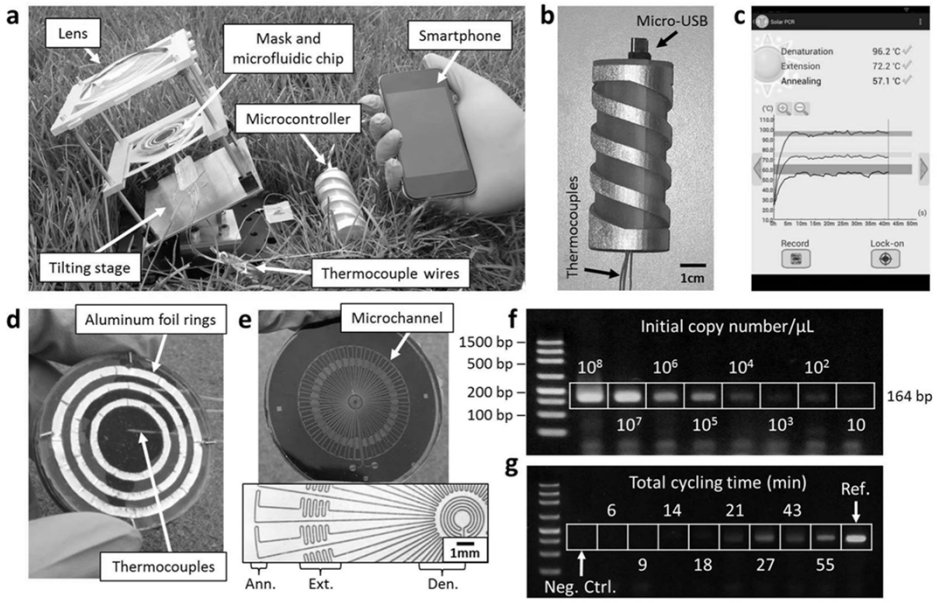
R. Gurrala[1], Z. Lang[2], L. Shepherd[2], D. Davidson[2], E. Harrison[2], M. McClure[1], S. Kaye[1], C. Toumazou[2,3] & G. S. Cooke[1]

The timely detection of viremia in HIV-infected patients receiving antiviral treatment is key to ensuring effective therapy and preventing the emergence of drug resistance. In high HIV burden settings, the cost and complexity of diagnostics limit their availability. We have developed a novel complementary metal-oxide semiconductor (CMOS) chip based, pH-mediated, point-of-care HIV-1 viral load monitoring assay that simultaneously amplifies and detects HIV-1 RNA. A novel low-buffer HIV-1 pH-LAMP (loop-mediated isothermal amplification) assay was optimised and incorporated into a pH sensitive CMOS chip. Screening of 991 clinical samples (164 on the chip) yielded a sensitivity of 95% (*in vitro*) and 88.8% (on-chip) at >1000 RNA copies/reaction across a broad spectrum of HIV-1 viral clades. Median time to detection was 20.8 minutes in samples with >1000 copies RNA. The sensitivity, specificity and reproducibility are close to that required to produce a point-of-care device which would be of benefit in resource poor regions, and could be performed on an USB stick or similar low power device.

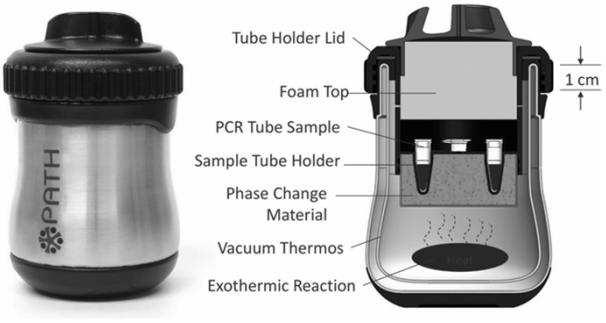


No electricity ??

Solar thermal PCR system



Sci Rep 2014;4:4137.



PATH NINA heater

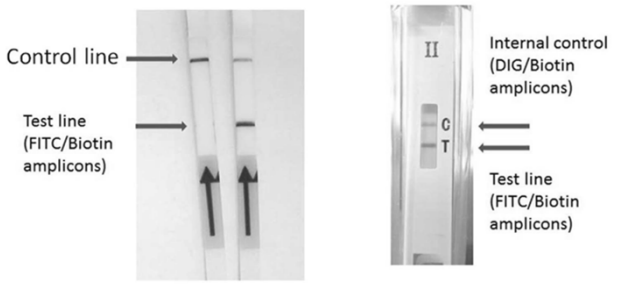
low-cost, electricity-free heater using an exothermic reaction thermally coupled with a phase change material

thermal standard deviation <0.5°C at operating temperature

a cost of approximately 0.06 USD per test for heater reaction materials

A. Milenia strips

B. Best II cassettes



HIV LAMP amplicon detection via Milenia test strips

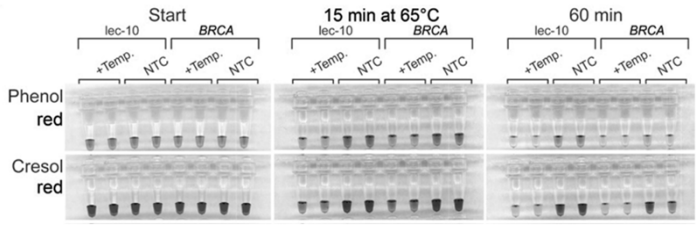
PLoS One 2014;9:e113693.

Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes

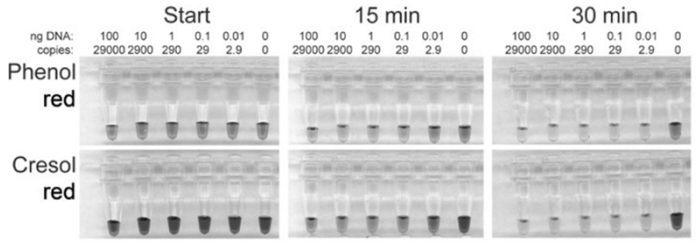
Nathan A. Tanner, Yinhua Zhang, and Thomas C. Evans Jr.
DNA Enzymes Division, New England Biolabs, Ipswich, MA *BioTechniques* 58:59-68 (February 2015)

rapid (<30 min) and sensitive (<10 copies) visual detection of amplified products using pH-sensitive dyes with minimal buffering capacity achieved with loop-mediated isothermal amplification (LAMP)

specificity



sensitivity



High sensitivity, loop-mediated isothermal amplification combined with colorimetric gold-nanoparticle probes for visual detection of high risk human papillomavirus genotypes 16 and 18

Ratchanida Kumvongpin[a], Patcharee Jearanaikool[a], Chotechana Wilailuckana[a], Nattaya Sae-ung[a], Prinya Prasongdee[a], Sakda Daduang[c], Metee Wongsena[d], Patcharee Boonsiri[e], Wansika Kiatpathomchai[f], Sukumarn Sanersak Swangvaree[g], Alisa Sandee[h], Jureerut Daduang[a,b,*]

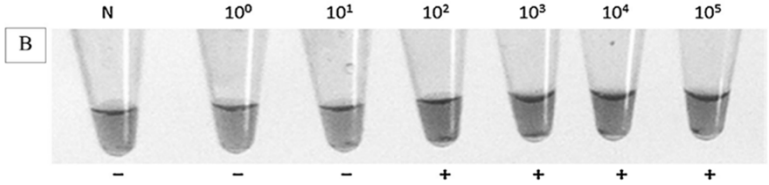
J Virol Methods 2016;234:90-5

gold nanoparticles (AuNP) attached to a single-stranded DNA probe for HPV16 and HPV18

LAMP incubation time of 20 min and a temperature of 65°C

detection of the LAMP product by AuNP color change

after LAMP amplification its products were hybridized with the AuNP probe for 5 min and then detected by the addition of magnesium salt



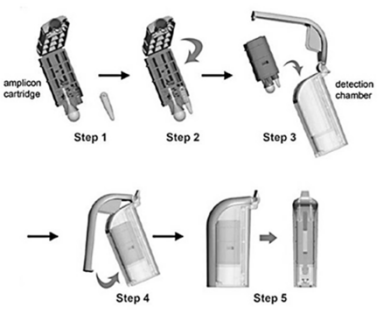
No electricity ??

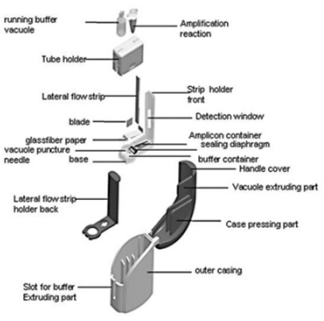
No instrument ???

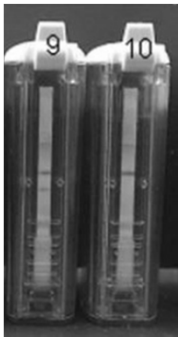
Ustar Biotechnologies (Hangzhou, China)

Cross Priming Amplification technology developed by Qimin You, while conducting research in Canada & US

- instrument free specimen processing
- isothermal nucleic acid amplification
- visual read-out detection and easy data interpretation
- cross contamination prevention
- glassified reagents for ambient temperature transport and storage





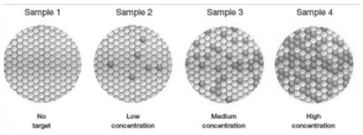


The Journal of Infectious Diseases 2010; 201(S1):S65–S71

revolution ?

Digital PCR

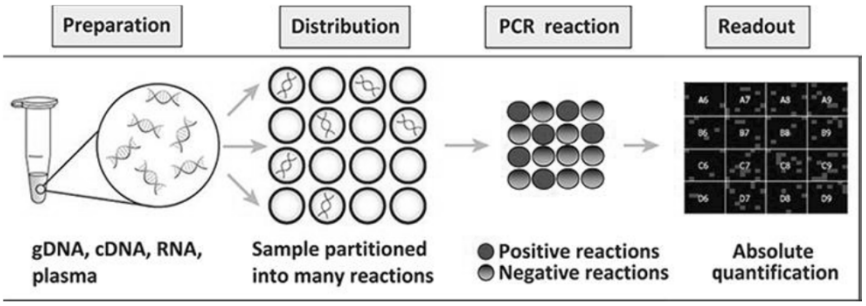
Digital PCR



new approach to nucleic acid detection and quantification

unlike real-time quantitative PCR, quantifies DNA without the need for a standard curve

provides precise absolute quantification of nucleic acids



Digital PCR

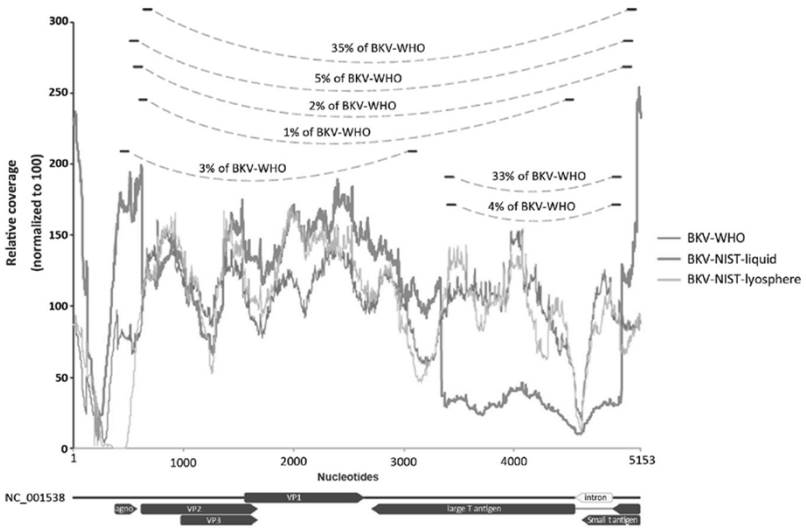
Life Technologies - QuantStudio 3D/12K

RainDance - RainDrop Digital PCR

Fluidigm - BioMark HD

Bio-Rad - QX100 ddPCR

Next-generation sequencing coverage map of BK virus WHO international standard and NIST materials



- the WHO BK standard is a mixed population of viruses, many of which have deletion of the T region
- qPCR assays targeting most of the T antigen will overestimate viral load approximately 4-fold

Bateman AC at al. Clin Chem 2017; 63:761-769

revolution ?

next generation sequencing

(diagnostic purposes)

Next generation sequencing

has the potential to dramatically revolutionize clinical microbiology

ultimate pathogen multiplex assay

identification of any expected or unexpected pathogens from single specimen

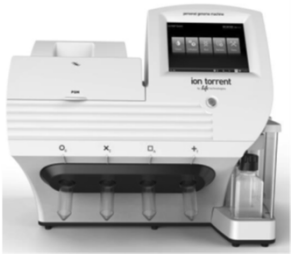
identification of rare pathogens not frequently on differential

identification of novel, highly divergent pathogens from a sample (metagenomics)

detection of virulence determinants and genetic markers/variants of drug resistance

tracking infectious disease outbreaks

Next-generation desktop sequencers



Ion PGM

NextSeq

miSeq

miniSeq

Ion Proton

Ion S5

MiniION

just because we can…

does not mean we should

Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak

Science 2014;345:1369-72

In its largest outbreak, Ebola virus disease is spreading through Guinea, Liberia, Sierra Leone, and Nigeria. We sequenced 99 Ebola virus genomes from 78 patients in Sierra Leone to ~2,000x coverage. We observed a rapid accumulation of interhost and intrahost genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from Middle African lineages ~2004, crossed from Guinea to Sierra Leone in May 2014, and has exhibited sustained human-to-human transmission subsequently, with no evidence of additional zoonotic sources. Since many of the mutations alter protein sequences and other biologically meaningful targets, they should be monitored for impact on diagnostics, vaccines, and therapies critical to outbreak response.

Whole-Genome Sequencing Shows That Patient-to-Patient Transmission Rarely Accounts for Acquisition of *Staphylococcus aureus* in an Intensive Care Unit

Clin Infect Dis 2014;58:609-18

James R. Price,[1] Tanya Golubchik,[2] Kevin Cole,[3] Daniel J. Wilson,[4,5] Derrick W. Crook,[4,6] Guy E. Thwaites,[7] Rory Bowden,[5] A. Sarah Walker,[4,6] Timothy E. A. Peto,[4,6] John Paul,[1,3] and Martin J. Llewelyn[1,8]

unselected patients admitted to an adult intensive care unit (ICU) were serially screened for *S. aureus*

all available isolates (n = 275) were *spa* typed and underwent whole-genome sequencing to investigate their relatedness at high resolution

Staphylococcus aureus was carried by 185 of 1109 patients sampled within 24 hours of ICU admission (16.7%); 59 (5.3%) patients carried MRSA

only a minority of *S. aureus* acquisitions can be explained by patient-to-patient transmission

whole-genome sequencing provides the resolution to disprove transmission events indicated by conventional methods and reveal unsuspected transmission events

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

Wilson MR et al., NEJM 2014; 370:2408-2417

14-year-old boy with severe combined immunodeficiency

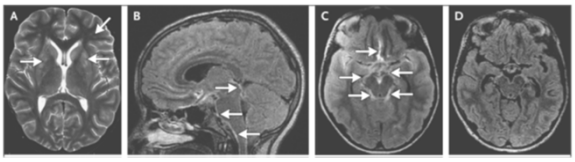
status epilepticus necessitating a medically induced coma

diagnostic workup including brain biopsy was unrevealing

clinical assays for leptospirosis negative

unbiased next-generation sequencing of the cerebrospinal fluid identified 475 of 3,063,784 sequence reads (0.016%) corresponding to leptospira infection

targeted antimicrobial agents were administered, patient was discharged home 32 days later with a status close to his premorbid condition



antibiotic susceptibility testing

??????????????

genomic

targeted assays

next-generation sequencing

alternative assays

phenotypic

revolution ?

genomic antimicrobial susceptibility testing

(targeted assays)

Genomic antibiotic susceptibility testing

nucleic acid amplification-mediated detection of resistance genes or mutations that are correlated with resistance to antibiotics plays an important role in clinical microbiology laboratories and will continue to do so

molecular testing will evolve versus syndrome-oriented multiplexed detection of pathogens including genomic AST

commercial competition will increase, prices per test will go down and in the end all tests will be of the "sample in – result out" format

significant part of antimicrobial susceptibility testing in the near future will be genomic

Detection of Isoniazid-, Fluoroquinolone-, Amikacin-, and Kanamycin-Resistant Tuberculosis in an Automated, Multiplexed 10-Color Assay Suitable for Point-of-Care Use

J Clin Microbiol 2017;55:183-198

Soumitesh Chakravorty,[a] Sandy S. Roh,[a] Jennifer Glass,[b] Laura E. Smith,[a]

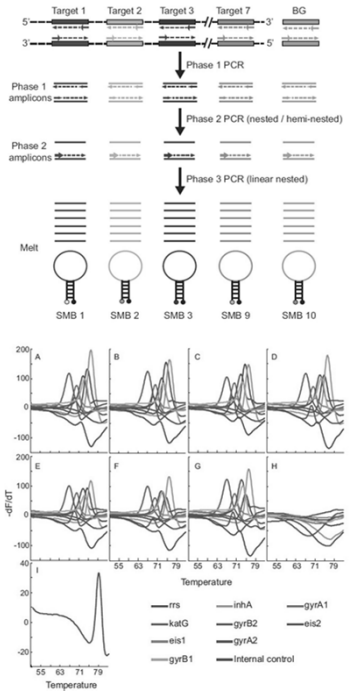
filter-based cartridge with an integrated sample processing function; testing directly from sputum

INOVATIONS:
- four new large-Stokes-shift fluorophores developed
- 10-color probe detection in a single PCR tube
- a new three-phase, double-nested PCR approach
- newly designed sloppy molecular beacons

32 commonly occurring mutant sequences tested in gyrA, gyrB, katG, and rrs genes and the promoters of inhA and eis genes responsible for resistance to isoniazid (INH), fluoroquinolone (FQ) drugs, amikacin (AMK), and kanamycin (KAN)

the rate of detection of heteroresistance equivalent to that by Sanger sequencing

compared to the results of phenotypic susceptibility testing, the sensitivity of the assay was 75% for FQs and 100% each for INH, AMK, and KAN and the specificity was 100% for INH and FQ and 94% for AMK and KAN



revolution ?

next generation sequencing

(antimicrobial resistant testing)

Review

Clinical Microbiology and Infection 23 (2017) 2–22

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

M.J. Ellington [1, †], O. Ekelund [2, †], F.M. Aarestrup [3], R. Canton [4], M. Doumith [1], C. Giske [5], H. Grundman [6], H. Hasman [7], M.T.G. Holden [8], K.L. Hopkins [1], J. Iredell [9], G. Kahlmeter [2], C.U. Köser [10], A. MacGowan [11], D. Mevius [12, 13], M. Mulvey [14], T. Naas [15], T. Peto [16], J.-M. Rolain [17], Ø. Samuelsen [18], N. Woodford [1, *]

For most bacterial species the major limitations to widespread adoption for whole genome sequencing (WGS) - based antibacterial antimicrobial susceptibility testing (AST) in clinical laboratories remain the current high-cost and limited speed of inferring antimicrobial susceptibility from WGS data as well as the dependency on previous culture because analysis directly on specimens remains challenging.

For most bacterial species there is currently insufficient evidence to support the use of WGS-inferred AST to guide clinical decision making.

WGS-AST should be a funding priority if it is to become a rival to phenotypic AST.

Next generation sequencing

routine clinical testing will be a reality with time; technology will improve

need for development of simplified solutions for all phases of testing

- sample preparation
- sequencing
- data analysis
- result interpretation

need to address clinical relevance of finding a fragment of nucleic acid that may not correlate with disease; detailed clinical evaluation and health-economics studies needed; better control of contamination needed

routine use will require well-vetted databases, rigorous quality assurance and quality control

revolution ?

molecular antimicrobial resistance testing

(alternative approaches)

A General Method for Rapid Determination of Antibiotic Susceptibility and Species in Bacterial Infections

J Clin Microbiol 2015;53:425-32

Anja Mezger,[a] Erik Gullberg,[b] Jenny Göransson,[c] Anna Zorzet,[b]* David Herthnek,[a] Eva Tano,[d] Mats Nilsson,[a] Dan I. Andersson[b]

Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Solna, Sweden[a]; Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden[b]; Q-linea AB, Uppsala, Uppsala, Sweden[c]; Department of Medical Sciences/Section of Clinical Bacteriology, Uppsala University, Uppsala, Sweden[d]

rapid identification of the bacterial species and simultaneous determination of their antibiotic susceptibility profiles

initial short cultivation step in the absence and presence of different antibiotics combined with sensitive species-specific padlock probe detection of the bacterial target DNA to allow a determination of growth (i.e. resistance) and no growth (i.e. susceptibility)

a proof-of-concept study:

- urinary tract infections
- antibiotic susceptibility profiles of *Escherichia coli* for ciprofloxacin and trimethoprim
- 100% accuracy in 3.5 h

revolution ?

New emerging technologies for phenotypic antimicrobial susceptibility testing

Accelerate *Pheno* System

ID and AST direct from positive blood culture

US FDA approved Accelerate Pheno system and Accelerate PhenoTest BC kit for ID and AST testing of pathogens directly from positive blood culture samples on 23 Feb 2017

Indicated for AST of pathogenic bacteria most commonly associated with bacteremia/sepsis

New emerging technologies with great potential for phenotypic antibiotic susceptibility testing

resonate mass measurement

microbial cell weighing by vibrating cantilevers + atomic force microscopy

isothermal microcalorimetry

asynchronous magnetic bead rotation

testing in microdroplets + epifluorescence

digital time-lapse microscopy

time-lapse single-cell imaging (SCMA)

high-throughput nanowell antibiotic susceptibility testing

forward laser light scatter technology

phase-shift reflectometric interference spectroscopy + micropillar architectures

gradient-generating microfluidic AST devices – chip based

gradient-generating microfluidic AST devices – hydrogel based

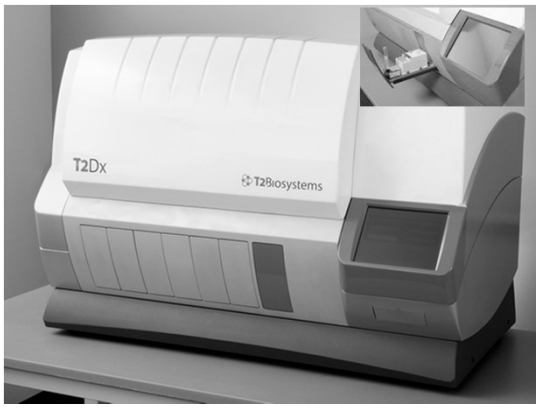
AST methods based on bacterial death

revolution ?

Direct detection and identification of bacteria using non-molecular, non MALDI-TOF technologies

T2 Biosystems (Lexington, MA)

- magnetic resonance technology (supermagnetic nanoparticles coated with target-specific binding agents cluster around the target, altering water molecules and their T2 relaxation signal)
- detects DNA, cells, proteins directly from specimens without extraction or amplification
- a low limit of detection (1-3 CFU/ml vs. 100-1000 CFU/ml for PCR)
- not impacted by the presence of antimicrobials
- printer-size detection device



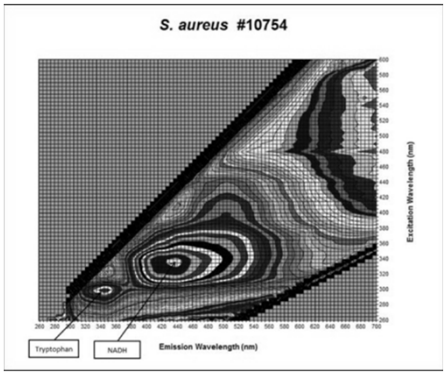
Rapid Intrinsic Fluorescence Method for Direct Identification of Pathogens in Blood Cultures

mBio 2013;4:e00865-13

John D. Walsh,[a] Jay M. Hyman,[a] Larisa Borzhemskaya,[a,b] Ann Bowen,[a,b] Caroline McKellar,[a] Michael Ullery,[c] Erin Mathias,[c] Christopher Ronsick,[d] John Link,[d] Mark Wilson,[d] Bradford Clay,[e] Ron Robinson,[e] Thurman Thorpe,[b] Alex van Belkum,[f] W. Michael Dunne, Jr[b]

intrinsic fluorescence spectroscopy of whole cells

multistage algorithm correctly classified 99.6% of unknown samples to the Gram level, 99.3% to the family level, and 96.5% to the species level



revolution ?

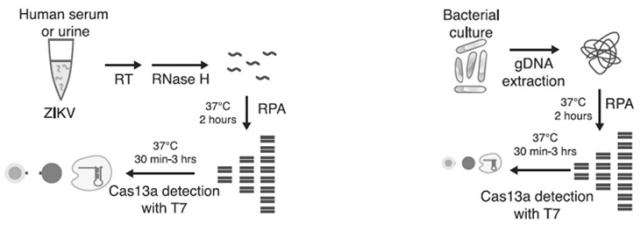
CRISPR-Cas – based diagnostic assays

Nucleic acid detection with CRISPR-Cas13a/C2c2

Science 2017; 356: 438–442

Jonathan S. Gootenberg,[1,2,3,4,5*] Omar O. Abudayyeh,[1,2,3,4,6*] Jeong Wook Lee,[7] Patrick Essletzbichler,[1,2,3,4] Aaron J. Dy,[1,4,8] Julia Joung,[1,2,3,4] Vanessa Verdine,[1,2,3,4] Nina Donghia,[7] Nichole M. Daringer,[8] Catherine A. Freije,[1,9] Cameron Myhrvold,[1,9] Roby P. Bhattacharyya,[1] Jonathan Livny,[1] Aviv Regev,[1,10] Eugene V. Koonin,[11] Deborah T. Hung,[1] Pardis C. Sabeti,[1,9,12,13] James J. Collins,[1,4,6,7,8†] Feng Zhang[1,2,3,4†]

Rapid, inexpensive, and sensitive nucleic acid detection may aid point-of-care pathogen detection, genotyping, and disease monitoring. The RNA-guided, RNA-targeting clustered regularly interspaced short palindromic repeats (CRISPR) effector Cas13a (previously known as C2c2) exhibits a "collateral effect" of promiscuous ribonuclease activity upon target recognition. We combine the collateral effect of Cas13a with isothermal amplification to establish a CRISPR-based diagnostic (CRISPR-Dx), providing rapid DNA or RNA detection with attomolar sensitivity and single-base mismatch specificity. We use this Cas13a-based molecular detection platform, termed Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK), to detect specific strains of Zika and Dengue virus, distinguish pathogenic bacteria, genotype human DNA, and identify mutations in cell-free tumor DNA. Furthermore, SHERLOCK reaction reagents can be lyophilized for cold-chain independence and long-term storage and be readily reconstituted on paper for field applications.



revolution ?

non-microorganism detection based

molecular diagnostic approaches

(host response diagnostics)

Non-microorganism detection based

molecular diagnostic approach ?

(i)

direct detection of the presence of microorganism(s)
in clinical specimens

determination of gene expression patterns in patient's blood
mononuclear cells specific for particular microorganism(s)

A Host-Based RT-PCR Gene Expression Signature to Identify Acute Respiratory Viral Infection

Sci Transl Med 2013;5:203ra126

Aimee K. Zaas[1,2], Thomas Burke[1], Minhua Chen[3], Micah McClain[1,2], Bradly Nicholson[4], Timothy Veldman[1], Ephraim L. Tsalik[1,2,4], Vance Fowler[2], Emanuel P. Rivers[5], Ronny Otero[5], Stephen F. Kingsmore[6], Deepak Voora[1,2], Joseph Lucas[1], Alfred O. Hero[7], Lawrence Carin[8], Christopher W. Woods[1,2,4,*], and Geoffrey S. Ginsburg[1,2,*]

102 adults vs. 41 healthy volunteers
sensitivity 89%
specificity 94%

Host gene expression classifiers diagnose acute respiratory illness etiology

Sci Transl Med 2016;8:322ra11

Ephraim L. Tsalik,[1,2,3]* Ricardo Henao,[1,4]* Marshall Nichols,[1] Thomas Burke,[1] Emily R. Ko,[1,5] Micah T. McClain,[1,3,6] Lori L. Hudson,[1] Anna Mazur,[1] Debra H. Freeman,[1,3] Tim Veldman,[1] Raymond J. Langley,[7] Eugenia B. Quackenbush,[8] Seth W. Glickman,[8] Charles B. Cairns,[8,9] Anja K. Jaehne,[10] Emanuel P. Rivers,[10] Ronny M. Otero,[10] Aimee K. Zaas,[1,3] Stephen F. Kingsmore,[11] Joseph Lucas,[1] Vance G. Fowler Jr.,[3] Lawrence Carin,[1,4] Geoffrey S. Ginsburg,[1†] Christopher W. Woods[1,3,6†]

overall accuracy
87% - 238/273
concordant with
clinical adjudication

Superiority of Transcriptional Profiling Over Procalcitonin for Distinguishing Bacterial From Viral Lower Respiratory Tract Infections in Hospitalized Adults

J Infect Dis 2015;212:213-22

Nicolas M. Suarez,[1,2] Eleonora Bunsow,[1,2] Ann R. Falsey,[3,4] Edward E. Walsh,[3,4] Asuncion Mejias,[1,2] and Octavio Ramilo[1,2]

[1]Center for Vaccines and Immunity, and [2]Division of Pediatric Infectious Diseases, The Research Institute at Nationwide Children's Hospital, and The Ohio State University College of Medicine, Columbus; [3]Department of Medicine, University of Rochester, and [4]Rochester General Hospital, New York

118 patients
sensitivity 95%
specificity 92%

Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children

JAMA 2016;316:835-45

Jethro A. Herberg, PhD; Myrsini Kaforou, PhD; Victoria J. Wright, PhD; Hannah Shailes, BSc; Hariklia Eleftherohorinou, PhD; Clive J. Hoggart, PhD; Miriam Cebey-López, MSc; Michael J. Carter, MRCPCH; Victoria A. Janes, MD; Stuart Gormley, MRes; Chisato Shimizu, MD; Adriana H. Tremoulet, MD; Anouk M. Barendregt, BSc; Antonio Salas, PhD; John Kanegaye, MD; Andrew J. Pollard, PhD; Saul N. Faust, PhD; Sanjay Patel, FRCPCH; Taco Kuijpers, PhD; Federico Martinón-Torres, PhD; Jane C. Burns, MD; Lachlan J. M. Coin, PhD; Michael Levin, FRCPCH; for the IRIS Consortium

Febrile children presenting to participating hospitals in the United Kingdom, Spain, the Netherlands, and the United States between 2009-2013.

A 2-transcript RNA expression signature distinguishing bacterial infection from viral infection was evaluated against clinical and microbiological diagnosis.

The discovery group of 240 children (median age, 19 months; 62%male) included 52 with definite bacterial infection, of whom 36 (69%) required intensive care, and 92 with definite viral infection, of whom 32 (35%) required intensive care.

Analysis of RNA expression data identified a 38-transcript signature distinguishing bacterial from viral infection. A smaller 2-transcript signature (FAM89A and IFI44L) was identified by removing highly correlated transcripts.

All 23 patients with microbiologically confirmed definite bacterial infection were classified as bacterial (sensitivity, 100% [95%CI, 100%-100%]) and 27 of 28 patients with definite viral infection were classified as viral (specificity, 96.4% [95%CI, 89.3%-100%]).

When applied to additional validation datasets from patients with meningococcal and inflammatory diseases, bacterial infection was identified with a sensitivity of 91.7%(95%CI, 79.2%-100%) and 90.0%(95%CI, 70.0%-100%), respectively, and with specificity of 96.0% (95%CI, 88.0%-100%) and 95.8% (95%CI, 89.6%-100%).

Diagnosis of Childhood Tuberculosis and Host RNA Expression in Africa

N Engl J Med 2014;370:1712-23.

culture-confirmed tuberculosis vs.
culture-negative tuberculosis, diseases other than tuberculosis, latent tuberculosis

51-transcript signature identified that distinguishing tuberculosis from other diseases in the South African and Malawian children

a risk score based on the signature for tuberculosis and for diseases other than tuberculosis showed a sensitivity of 82.9% (68.6 to 94.3) and a specificity of 83.6% (74.6 to 92.7) for the diagnosis of culture-confirmed tuberculosis

the sensitivity of the Xpert MTB/RIF assay for molecular detection of *M. tuberculosis* DNA in cases of culture-confirmed tuberculosis was 54.3% (37.1 to 68.6), specificity 100%

RNA expression signatures provided data that helped distinguish tuberculosis from other diseases in African children with and those without HIV infection

Non-microorganism detection based molecular diagnostic approach ?
(ii)

direct detection of the presence of microorganism(s)
in clinical specimens

uses deep sequencing to monitor gene expression at the level of translation
rather than transcription and/or complex protein analysis
(sequencing a cDNA library derived from the short fragments of mRNA covered by the ribosome)

providing novel insights into the identities and amounts of proteins being
produced in cells infected with microorganism(s)

Decoding Viral Infection by Ribosome Profiling

J Virology 2015; 89: 6164-6166

Noam Stern-Ginossar
Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

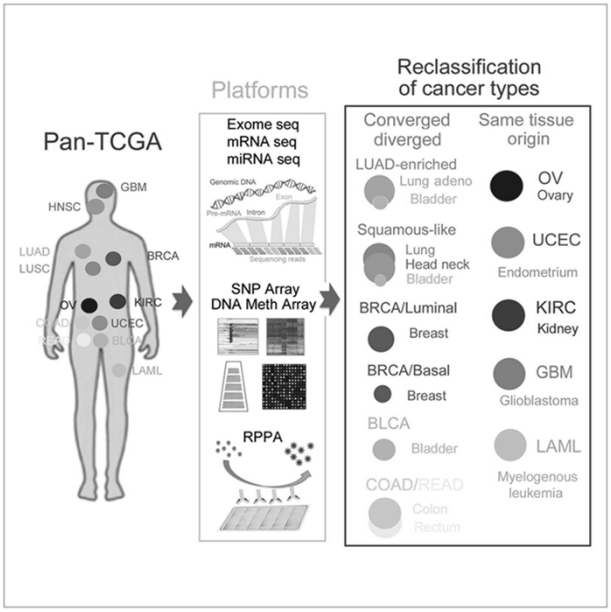
Non-microorganism detection based molecular diagnostic approach ?

(iii)

direct detection of the presence of microorganism(s)
in clinical specimens

treatment of infectious diseases with non-traditional drugs, compounds which were originally not developed as antimicrobial agents

Categorising tumours by the genetic and epigenetic changes in their cells, rather than by anatomy and histology



Cell (2014), http://dx.doi.org/10.1016/j.cell.2014.06.049

Host-Directed Antimicrobial Drugs with Broad-Spectrum Efficacy against Intracellular Bacterial Pathogens

Daniel M. Czyż,[a,b] Lakshmi-Prasad Potluri,[a,b*] Neeta Jain-Gupta,[a,c] Sean P. Riley,[a,b*] Juan J. Martinez,[a,b*] Theodore L. Steck,[c] Sean Crosson,[a,c] Howard A. Shuman,[a,b] Joëlle E. Gabay[b]

Howard Taylor Ricketts Laboratory, University of Chicago, Argonne National Laboratory, Lemont, Illinois, USA[a]; Department of Microbiology, University of Chicago, Chicago, Illinois, USA[b]; Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois, USA[c]

* Present address: Lakshmi-Prasad Potluri, Biology Department, University of Nebraska at Omaha, Omaha, Nebraska, USA; Sean P. Riley and Juan J. Martinez, Vector-Borne Disease Laboratory, Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, Louisiana, USA.

ABSTRACT We sought a new approach to treating infections by intracellular bacteria, namely, by altering host cell functions that support their growth. We screened a library of 640 Food and Drug Administration (FDA)-approved compounds for agents that render THP-1 cells resistant to infection by four intracellular pathogens. We identified numerous drugs that are not antibiotics but were highly effective in inhibiting intracellular bacterial growth with limited toxicity to host cells. These compounds are likely to target three kinds of host functions: (i) G protein-coupled receptors, (ii) intracellular calcium signals, and (iii) membrane cholesterol distribution. The compounds that targeted G protein receptor signaling and calcium fluxes broadly inhibited *Coxiella burnetii*, *Legionella pneumophila*, *Brucella abortus*, and *Rickettsia conorii*, while those directed against cholesterol traffic strongly attenuated the intracellular growth of *C. burnetii* and *L. pneumophila*. These pathways probably support intracellular pathogen growth so that drugs that perturb them may be therapeutic candidates. Combining host- and pathogen-directed treatments is a strategy to decrease the emergence of drug-resistant intracellular bacterial pathogens.

IMPORTANCE Although antibiotic treatment is often successful, it is becoming clear that alternatives to conventional pathogen-directed therapy must be developed in the face of increasing antibiotic resistance. Moreover, the costs and timing associated with the development of novel antimicrobials make repurposed FDA-approved drugs attractive host-targeted therapeutics. This paper describes a novel approach of identifying such host-targeted therapeutics against intracellular bacterial pathogens. We identified several FDA-approved drugs that inhibit the growth of intracellular bacteria, thereby implicating host intracellular pathways presumably utilized by bacteria during infection.

mBio 2014; 5:e01534-14

Non-microorganism detection based

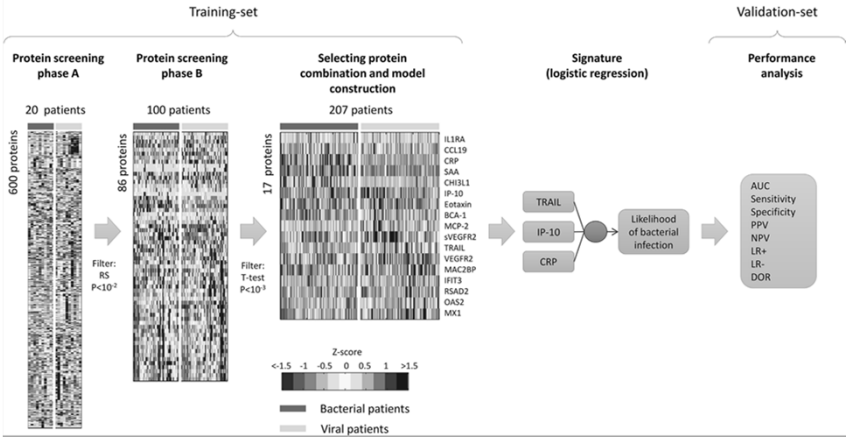
<u>non-molecular</u> diagnostic approach ?

(iv)

A Novel Host-Proteome Signature for Distinguishing between Acute Bacterial and Viral Infections

PLoS ONE 2015;10:e0120012

Kfir Oved[1]*, Asi Cohen[1], Olga Boico[1], Roy Navon[1], Tom Friedman[1,2], Liat Etshtein[1,3], Or Kriger[1¤a], Ellen Bamberger[1,3,5], Yura Fonar[1¤b], Renata Yacobov[4], Ron Wolchinsky[6], Galit Denkberg[7], Yaniv Dotan[3,8], Amit Hochberg[4], Yoram Reiter[6], Moti Grupper[3,9], Isaac Srugo[3,5], Paul Feigin[10], Malka Gorfine[10], Irina Chistyakov[3,5], Ron Dagan[11], Adi Klein[4], Israel Potasman[3,9], Eran Eden[1]*



host-proteins measured using ELISA, Luminex, protein-arrays and flow-cytometry → ImmunoXpert

A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study

Chantal B van Houten, Joris A H de Groot, Adi Klein, Isaac Srugo, Irena Chistyakov, Wouter de Waal, Clemens B Meijssen, Wim Avis, Tom F W Wolfs, Yael Shachor-Meyouhas, Michal Stein, Elisabeth A M Sanders, Louis J Bont

Lancet Infect Dis 2017;17: 431–40

Summary

Background A physician is frequently unable to distinguish bacterial from viral infections. ImmunoXpert is a novel assay combining three proteins: tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), interferon gamma induced protein-10 (IP-10), and C-reactive protein (CRP). We aimed to externally validate the diagnostic accuracy of this assay in differentiating between bacterial and viral infections and to compare this test with commonly used biomarkers.

Methods In this prospective, double-blind, international, multicentre study, we recruited children aged 2–60 months with lower respiratory tract infection or clinical presentation of fever without source at four hospitals in the Netherlands and two hospitals in Israel. A panel of three experienced paediatricians adjudicated a reference standard diagnosis for all patients (ie, bacterial or viral infection) using all available clinical and laboratory information, including a 28-day follow-up assessment. The panel was masked to the assay results. We identified majority diagnosis when two of three panel members agreed on a diagnosis and unanimous diagnosis when all three panel members agreed on the diagnosis. We calculated the diagnostic performance (ie, sensitivity, specificity, positive predictive value, and negative predictive value) of the index test in differentiating between bacterial (index test positive) and viral (index test negative) infection by comparing the test classification with the reference standard outcome.

Findings Between Oct 16, 2013 and March 1, 2015, we recruited 777 children, of whom 577 (mean age 21 months, 56% male) were assessed. The majority of the panel diagnosed 71 cases as bacterial infections and 435 as viral infections. In another 71 patients there was an inconclusive panel diagnosis. The assay distinguished bacterial from viral infections with a sensitivity of 86·7% (95% CI 75·8–93·1), a specificity of 91·1% (87·9–93·6), a positive predictive value of 60·5% (49·9–70·1), and a negative predictive value of 97·8% (95·6–98·9). In the more clear cases with unanimous panel diagnosis (n=354), sensitivity was 87·8% (74·5–94·7), specificity 93·0% (89·6–95·3), positive predictive value 62·1% (49·2–73·4), and negative predictive value 98·3% (96·1–99·3).

Interpretation This external validation study shows the diagnostic value of a three-host protein-based assay to differentiate between bacterial and viral infections in children with lower respiratory tract infection or fever without source. This diagnostic based on CRP, TRAIL, and IP-10 has the potential to reduce antibiotic misuse in young children.

small modification, great impact ?

Reduction in Blood Culture Contamination Through Use of Initial Specimen Diversion Device

Mark E. Rupp,[1] R. Jennifer Cavalieri,[1] Cole Marolf,[1] and Elizabeth Lyden[2]

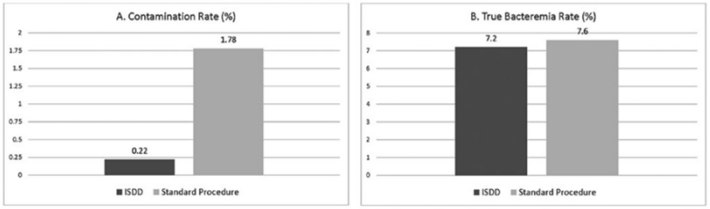
[1]Division of Infectious Diseases, and [2]Department of Epidemiology, University of Nebraska Medical Center, Omaha

Background. Blood culture contamination is a clinically significant problem that results in patient harm and excess cost.

Methods. In a prospective, controlled trial at an academic center Emergency Department, a device that diverts and sequesters the initial 1.5–2 mL portion of blood (which presumably carries contaminating skin cells and microbes) was tested against standard phlebotomy procedures in patients requiring blood cultures due to clinical suspicion of serious infection.

Results. In sum, 971 subjects granted informed consent and were enrolled resulting in 904 nonduplicative subjects with 1808 blood cultures. Blood culture contamination was significantly reduced through use of the initial specimen diversion device™ (ISDD) compared to standard procedure: (2/904 [0.22%] ISDD vs 16/904 [1.78%] standard practice, $P = .001$). Sensitivity was not compromised: true bacteremia was noted in 65/904 (7.2%) ISDD vs 69/904 (7.6%) standard procedure, $P = .41$. No needlestick injuries or potential bloodborne pathogen exposures were reported. The monthly rate of blood culture contamination for all nurse-drawn and phlebotomist-drawn blood cultures was modeled using Poisson regression to compare the 12-month intervention period to the 6 month before and after periods. Phlebotomists (used the ISDD) experienced a significant decrease in blood culture contamination while the nurses (did not use the ISDD) did not. In sum, 73% of phlebotomists completed a post-study anonymous survey and widespread user satisfaction was noted.

Conclusions. Use of the ISDD was associated with a significant decrease in blood culture contamination in patients undergoing blood cultures in an Emergency Department setting.

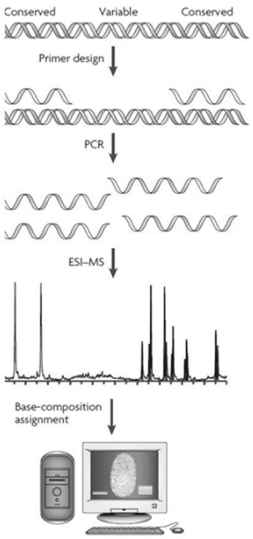


Great promises:

little (current) impact

electrospray ionization mass spectrometry (PCR/ESI-MS)

PLEX-ID (Abbott)



Improved Sensitivity for Molecular Detection of Bacterial and *Candida* Infections in Blood

J Clin Microbiol 2014;52:3164-74

Andrea Bacconi,[a] Gregory S. Richmond,[a] Michelle A. Baroldi,[a] Thomas G. Laffler,[a] Lawrence B. Blyn,[a] Heather E. Carolan,[a] Mark R. Frinder,[a] Donna M. Toleno,[a] David Metzgar,[a] Jose R. Gutierrez,[a] Christian Massire,[a] Megan Rounds,[a] Natalie J. Kennel,[a] Richard E. Rothman,[b] Stephen Peterson,[b] Karen C. Carroll,[c] Teresa Wakefield,[c] David J. Ecker,[a] Rangarajan Sampath[a]

Ibis Biosciences, Inc., Carlsbad, California, USA[a]; Department of Emergency Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA[b]; The Johns Hopkins Hospital Clinical Microbiology Laboratory, Baltimore, Maryland, USA[c]

PCR followed by electrospray ionization mass spectrometry (PCR/ESI-MS)

new integrated specimen preparation that substantially improves the sensitivity

an efficient lysis method and automated system for processing 5 ml of whole blood

PCR amplification formulations optimized to tolerate high levels of human DNA

PCR/ESI-MS was 91% sensitive and 99% specific compared to culture

Great promises:

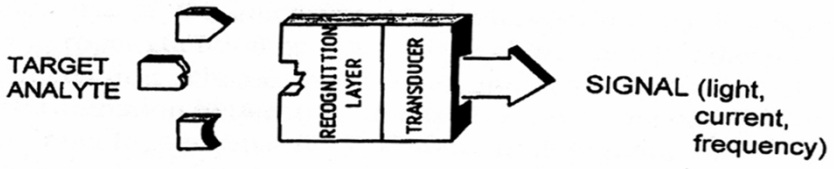
little (current) impact

biosensors

Biosensors

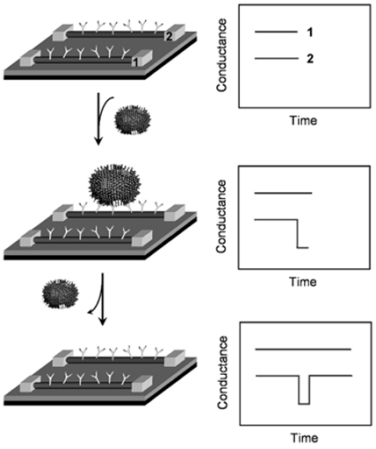
small devices which utilize biological reactions

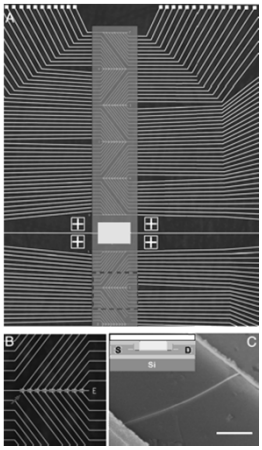
for detecting target analytes



BIOSENSOR

Patolsky F, Zheng G, Hayden O, Lakadamyali M, Zhuang X, Lieber CM.
Electrical detection of single viruses.
Proc Natl Acad Sci USA 2004; 101:14017-22.



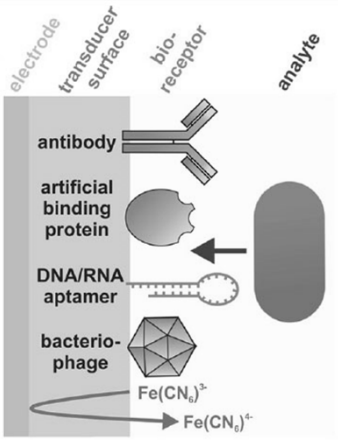


Biosensors for Whole-Cell Bacterial Detection

Clin Micro Rev 2014; 27:631-646

Asif Ahmed, Jo V. Rushworth,* Natalie A. Hirst, Paul A. Millner
School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

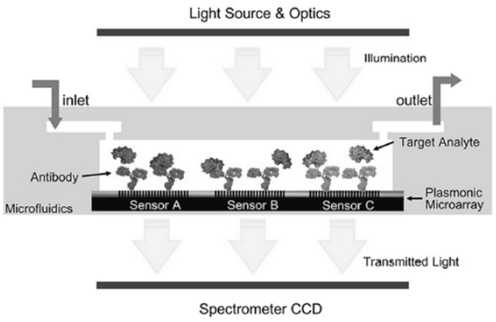
- Optical Biosensors
- Mechanical Biosensors
- Electrochemical Biosensors
  - Potentiometric sensors
  - Amperometric sensors
  - Impedimetric sensors

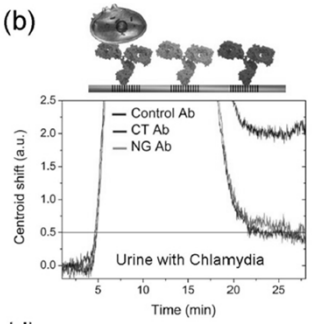


Multiplexed nanoplasmonic biosensor for one-step simultaneous detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine

Maria Soler[a], Alexander Belushkin[a], Andrea Cavallini[a], Carole Kebbi-Beghdadi[b], Gilbert Greub[b], Hatice Altug[a,*]

Biosensors and Bioelectronics 94 (2017) 560–567





gold nanohole sensor arrays that exhibit the extraordinary optical transmission providing highly sensitive analysis in a label-free configuration

detection and quantification of the bacteria in real-time

immunoassay (urine); LOD - 300 CFU/mL *C. trachomatis;* 1500 CFU/mL *N. gonorrhoeae*

Great (crazy) ideas, but…?

Noninvasive imaging of *Staphylococcus aureus* infections with a nuclease-activated probe

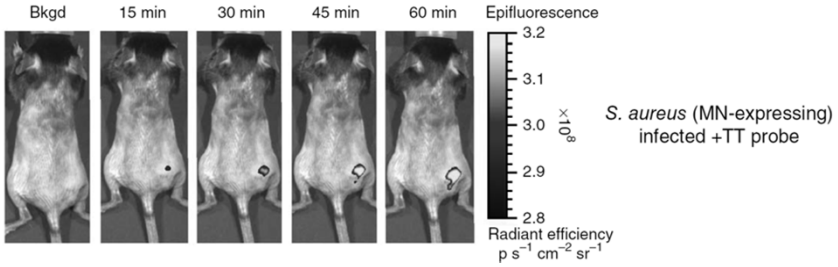
Nat Med 2014;20:301-6

Frank J Hernandez[1], Lingyan Huang[2], Michael E Olson[3], Kristy M Powers[2], Luiza I Hernandez[1], David K Meyerholz[4], Daniel R Thedens[5], Mark A Behlke[2], Alexander R Horswill[3] & James O McNamara II[1]

molecular real-time in vivo test - rapid localization of bacterial infections in living animals

molecular imaging approach for the specific, noninvasive detection of *S. aureus* based on the activity of the *S. aureus* secreted nuclease, micrococcal nuclease

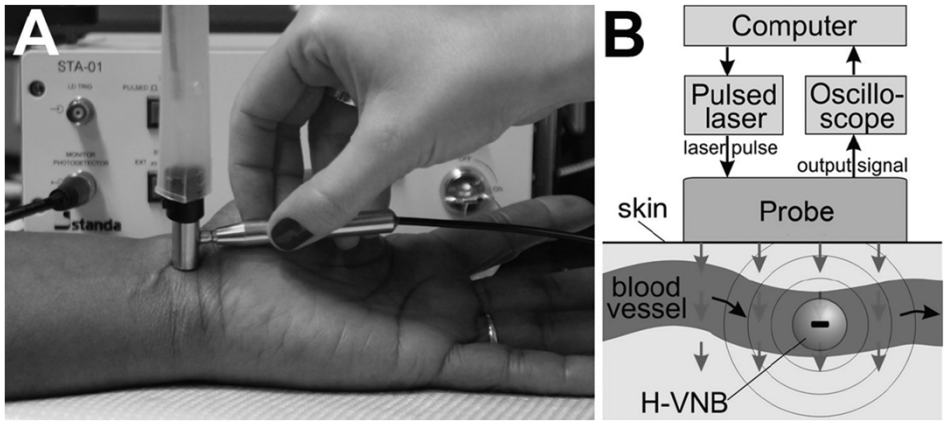
several short synthetic oligonucleotides, rendered resistant to mammalian nucleases by various chemical modifications and flanked with a fluorophore and quencher



Transdermal Diagnosis of Malaria Using Vapor Nanobubbles

Ekaterina Lukianova-Hleb, Sarah Bezek, Reka Szigeti, Alexander Khodarev, Thomas Kelley, Andrew Hurrell, Michail Berba, Nirbhay Kumar, Umberto D'Alessandro, Dmitri Lapotko

Emerg Infect Dis 2015;21:1122-7



20-second noninvasive diagnosis of *Plasmodium falciparum* infection without drawing blood or using any reagent

A Breath Fungal Secondary Metabolite Signature to Diagnose Invasive Aspergillosis

Clin Infect Dis 2014;59:1733-40

Sophia Koo,[1,2,3,a] Horatio R. Thomas,[1,3,a] S. David Daniels,[1] Robert C. Lynch,[1] Sean M. Fortier,[1] Margaret M. Shea,[1] Preshious Rearden,[4] James C. Comolli,[4] Lindsey R. Baden,[1,2,3] and Francisco M. Marty[1,2,3]

[1]Division of Infectious Diseases, Brigham and Women's Hospital, [2]Dana-Farber Cancer Institute, [3]Harvard Medical School, Boston, and [4]Draper Laboratory, Cambridge, Massachusetts

- thermal desorption-gas chromatography/mass spectrometry
- prospectively collected breath samples
- patients with proven or probable invasive aspergillosis vs. patients without aspergillosis

detection of α-trans-bergamotene, β-trans-bergamotene, a β-vatirenene–like sesquiterpene, or trans-geranylacetone identified patients with invasive aspergillosis with 94% sensitivity (95% CI, 81%-98%) and 93% specificity (95% CI, 79%-98%)

Diagnosis of Tuberculosis by Trained African Giant Pouched Rats and Confounding Impact of Pathogens and Microflora of the Respiratory Tract

Georgies F. Mgode,[a,b] Bart J. Weetjens,[c] Thorben Nawrath,[d] Christophe Cox,[c] Maureen Jubitana,[c] Rober Stéphan Cohen-Bacrie,[e] Marielle Bedotto,[e] Michel Drancourt,[e] Stefan Schulz,[d] and Stefan H. E. Kaufman

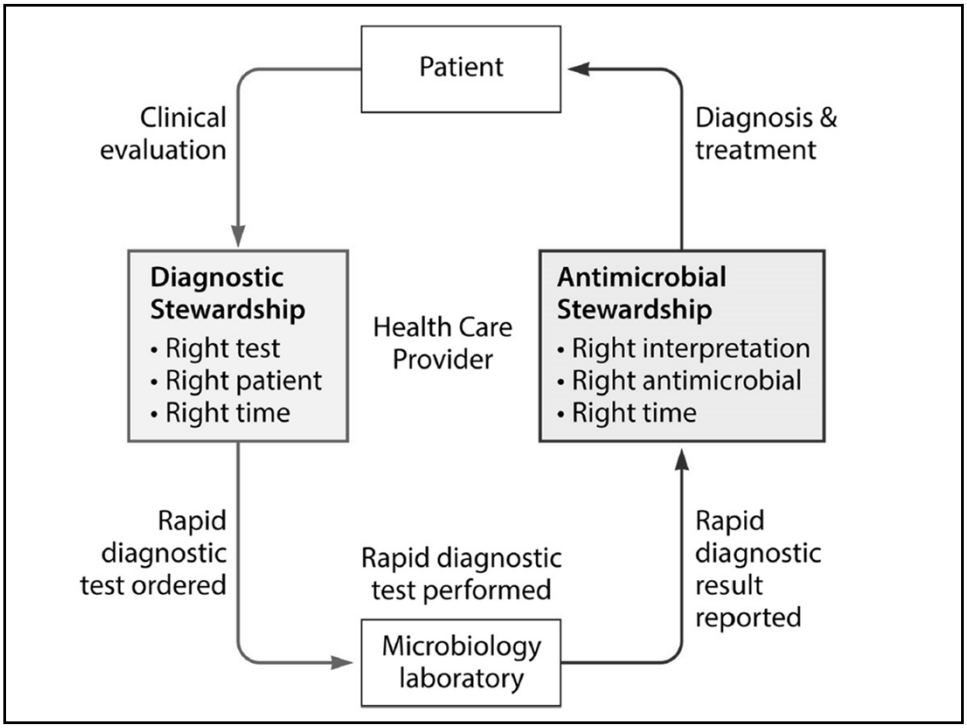
Department of Immunology, Max Planck Institute for Infection Biology, Campus Charité Mitte, Berlin, Germany[a]; Pest Management Agriculture, Chuo Kikuu, Morogoro, Tanzania[b]; Anti-Persoonmijnen Ontmijnende Product Ontwikelling (APOPO vzw), Antwerp, Belg Technische Universität Braunschweig, Braunschweig, Germany[d]; and URMITE UMR CNRS 6236, IRD 198, IFR48, IHU POLMIT, Universi France[e]

Trained African giant-pouched rats (*Cricetomys gambianus*) can detect *Mycobacterium tuberculosis* and show potential for the diagnosis of tuberculosis (TB). However, rats' ability to discriminate between clinical sputum containing other *Mycobacterium* spp. and nonmycobacterial species of the respiratory tract is unknown. It is also unknown whether nonmycobacterial species produce odor similar to *M. tuberculosis* and thereby cause the detection of smear-negative sputum. Sputum samples from 289 subjects were analyzed by smear microscopy, culture, and rats. *Mycobacterium* spp. were isolated on Lowenstein-Jensen medium, and nonmycobacterial species were isolated on four different media. The odor from nonmycobacterial species from smear- and *M. tuberculosis* culture-negative sputa detected by ≥2 rats ("rat positive") was analyzed by gas chromatography-mass spectrometry and compared to the *M. tuberculosis* odor. Rats detected 45 of 56 confirmed cases of TB, 4 of 5 suspected cases of TB, and 63 of 228 TB-negative subjects (sensitivity, 80.4%; specificity, 72.4%; accuracy, 73.9%; positive predictive value, 41.7%; negative predictive value, 93.8%). A total of 37 (78.7%) of 47 mycobacterial isolates were *M. tuberculosis* complex, with 75.7% from rat-positive sputa. Ten isolates were nontuberculous mycobacteria, one was *M. intracellulare*, one was *M. avium* subsp. *hominissuis*, and eight were unidentified. Rat-positive sputa with *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Staphylococcus* spp., and *Enterococcus* spp. were associated with TB. *Rhodococcus*, *Nocardia*, *Streptomyces*, *Staphylococcus*, and *Candida* spp. from rat-positive sputa did not produce *M. tuberculosis*-specific volatiles (methyl nicotinate, methyl *para*-anisate, and *ortho*-phenylanisole). Prevalence of *Mycobacterium*-related *Nocardia* and *Rhodococcus* in smear-negative sputa did not equal that of smear-negative mycobacteria (44.7%), of which 28.6% were rat positive. These findings and the absence of *M. tuberculosis*-specific volatiles in nonmycobacterial species indicate that rats can be trained to specifically detect *M. tuberculosis*.

Journal of Clinical Microbiology 2012; 50: 274-280

our technical capabilities are exceeding our ability to apply them effectively and economically to human problems

Bartlett RC, 1974



Diagnostic stewardship

The goal of diagnostic stewardship is to select the right test for the right patient, generating accurate, clinically relevant results at the right time to optimally influence clinical care and to conserve health care resources.

Goal	Key question	Key considerations and potential strategies
Right test	Is the test appropriate for the clinical setting?	Sensitivity and specificity Predictive values Testing volumes Diagnostic yield Laboratory feasibility Cost Clinical impact
Right patient	Will the clinical care of the patient be affected by the test result?	Laboratory test utilization committee Automatic laboratory reflex CPOE decision support Appropriate use criteria Indication selection Prior authorization Benchmarking Specimen rejection
Right time	Will the result be available in time to optimally affect care?	Time to specimen receipt Centralized vs point-of-care testing On-demand vs batched testing Specimen preparation time Run time Result reporting time

Messacar K at al. J Clin Microbiol 2017; 55:715–723.

Conclusions

the clinical microbiology laboratory is in the midst of a diagnostic revolution

continuous introduction of newer technologies and approaches over years

more rapid; more sensitive; lower cost?

adequate training needed; new skills needed; new profile of microbiologist needed?

several open quality and regulatory issues

more data on hard endpoints needed; integration into clinical care challenging

change management; project management; team management

continuous education and implementatoion of diagnostic and antimicrobial stewardship are necessary to ensure that new technologies conserve, rather than consume, additional health care resources and optimally affect patient care

Avtomatizacija bakteriološkega laboratorija

7. Likarjev simpozij
NOVI KONCEPTI V DIAGNOSTIČNI MIKROBIOLOGIJI
Ljubljana, 15. 06. 2017

Katja Seme
Inštitut za mikrobiologijo in imunologijo, UL MF

Clinical laboratory automation

the use of instruments and specimen processing equipment to perform clinical assays with only minimal involvement of technologist

Burtis CA, Ashwood ER, Bruns DE (Eds).
Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 3rd Ed. St Louis: Elsevier Saaunders, 2006

Historical impediments to automation in bacteriology

- bacteriology is too complex to automate
- no machine can replace a human in the bacteriology laboratory
- cost of automation
- bacteriology laboratories are too small for automation

Bourbeau PP, Ledeboer NA. Automation in clinical microbiology. J Clin Microbiol 2013; 51: 1658-65.

Culture based bacteriology

- specimen inoculation
- incubation
- reading
- identification
- AST

Automated blood culture systems

reduced the time to detect microbial growth by
24 to 36 hours
in comparison to manual blood culture systems

Bactec (BD, USA)

BacT/Alert (BioMerieux, France)

VersaTREK Blood Culture System (Thermo Scientific, USA)

Automated systems for identification of bacteria and yeast

Manufacturer	Instruments	Principle(s)	Panels[b]	Organisms in database (no. of taxa)	No. of tests	Incubation (h)	Software/expert systems
bioMérieux	Vitek 2 XL Vitek 2 60 Vitek 2 Compact 60 Vitek 2 Compact 30 Vitek 2 Compact 15	Colorimetric carbon source utilization; enzymatic activity; resistance	GP GN NH ANC Yeast	119 143 26 61 52	43	8	Advanced Expert System, Observa
Siemens	MicroScan WalkAway *plus*	Overnight panels turbidimetric detection of carbon source utilization; enzymatic activity Rapid panels use fluorometric detection of preformed enzymes	GN Convent. GN Rapid GN Synergies GP Convent. GP Rapid GP Synergies Yeast Anaerobe NH	116 139 139 51 53 53 42 54 20	34 36 27 36 27 25 18	2.5 16–18	LabPro
BD Diagnostics	BD Phoenix	Colorimetric and fluorometric detection	GP GN *Streptococcus* Yeast ID	140 161 27 64	48	8–16	BDXpert, BD EpiCenter
TREK Diagnostic Systems	ARIS	Fluorometric detection	GP GN	41 137	32 31	5–18	SWIN
Biolog	OmniLog	Carbon source utilization detection by reduction of tetrazolium violet	GP GN	2,500	95	4–24[c]	GEN III
MIDI, Inc.	Sherlock microbial identification system	Cell wall fatty acid analysis using gas chromatography	GP GN Yeast	1,500	NA	24[c]	CLIN 50 database

[a]Derived from: http://www.bd.com/ds/productCenter/IS.asp; www.biomerieux-usa.com; www.siemens.com/diagnostics; www.biolog.com; http://www.midi-inc.com/; http://www.trekds.com/products/sensititre/c_standardid.asp.
[b]GP, Gram positive; GN, Gram negative; NH, *Neisseria*, *Haemophilus*; ANC, anaerobe; Convent., conventional; ID, identification; NA, not applicable.
[c]Overnight growth on particular media is required; assay takes about 2 h to perform.

Manual of Clinical Microbiology, Vol 1, 11 Ed, 2015

Ongoing Revolution in Bacteriology: Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

Clinical Infectious Diseases 2009; 49:543–51

Piseth Seng,[a] Michel Drancourt,[a] Frédérique Gouriet, Bernard La Scola, Pierre-Edouard Fournier, Jean Marc Rolain, and Didier Raoult

MALDI Biotyper (Bruker Daltonik GmbH, Germany)

VitekMS (bioMérieux, France)

Advantages of MALDI-TOF

- speed (<3 min/isolate; 96 samples/h)
- easy to perform
- small amount of organism required
- database: > 2000 species entries
- direct ID from samples (positive blood cultures, urine)
- low cost

Automated broth microdilution susceptibility testing instruments

TABLE 2 Overview of automated broth microdilution susceptibility testing instrumentation[a]

Manufacturer	System(s)	Panel capacity	Panels	Types of panels (no.)	Instrument features	Software
Becton Dickinson	BD Phoenix	100	Two-sided panels: 85-well AST/51-well ID or 136-well AST (Emerge)	Gram pos (4) Gram neg (12) *Streptococcus* (1) Emerge Gram neg (1)	Automated adjustment of inoculum and AST dilution. AST panels available as MIC ± ID substrates. Turbidimetric and redox indicator readings every 20 min. Full-range MICs.	BDXpert BD EpiCenter
bioMérieux	VITEK 2, VITEK 2 XL	60, 120	64-well cards	Gram pos (2) Gram neg (14) *S. pneumoniae* (1) Yeast (1)	Automated AST dilution and filling/sealing of cards. Turbidimetric readings every 15 min. MICs derived from 1–6 antimicrobial agent dilutions.	AES Myla Observa
	VITEK 2 Compact	15, 30, 60	64-well cards	See VITEK 2	Less automated, more affordable than VITEK 2	See VITEK 2
Siemens	MicroScan WalkAway *plus*	40 or 96	Standard 96-microwell trays	ON (32) *Streptococcus* (1) ESBL (1) Rapid (4) Synergies *plus* (10)	Panels available as full-range MIC or breakpoint MIC. Combination panels include ID substrates. MIC readings: ON, turbidimetric; "read when ready," turbidimetric; rapid panels (3.5–15 h), fluorometric.	LabPro LabPro Alert
Thermo Scientific	Sensititre ARIS 2X	64	Standard 96-microwell trays	Gram pos (2) Gram neg (4) *Streptococcus* (1) ESBL (1) Yeast (1)	Fluorometric readings after ON incubation of full-range MIC trays. *Haemophilus*/*S. pneumoniae*, RUO (mycobacteria, anaerobic, campylobacter, Gram neg, yeast), and custom (frozen, dried) plates also available.	SWIN epidemiology module

[a]neg, negative; ON, overnight; pos, positive; RUO, research use only.

Manual of Clinical Microbiology, Vol 1, 11 Ed, 2015

Driving forces of change toward automation in bacteriology

- increase in sample number
- shortage of trained personnel
- limited budget
- growing demand for improved quality
- technical innovations:
  - introduction of liquid based swabs (liquid bacteriology)
  - emergence of MALDI-TOF

Bourbeau PP, Ledeboer NA. Automation in clinical microbiology. J Clin Microbiol 2013; 51: 1658-65.

Automated specimen processing

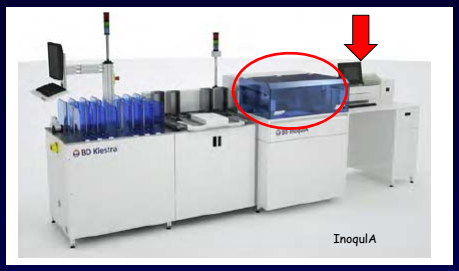
WASP (Copan, Italy)

InoqulA (BD Kiestra, The Netherlands)

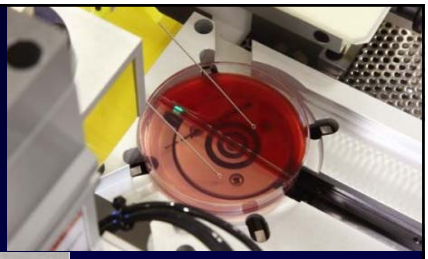
Previ Isola (bioMerieux, France)

PreLUD (i2a Diagnostics, France)

AUTOPLAK (NTE Healthcare, Spain)



WASP



InoqulA

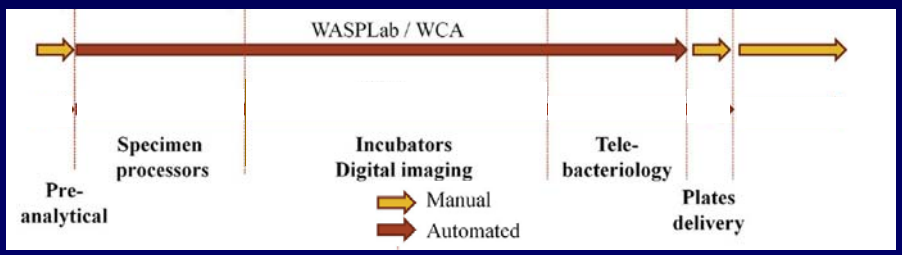
PREVI Isola

Partial laboratory automation

WASPLab (Copan, Italy)

Work Cell Automation (WCA) (BD Kiestra, The Netherlands)

PreLUD + Maestro (i2a Diagnostics, France)

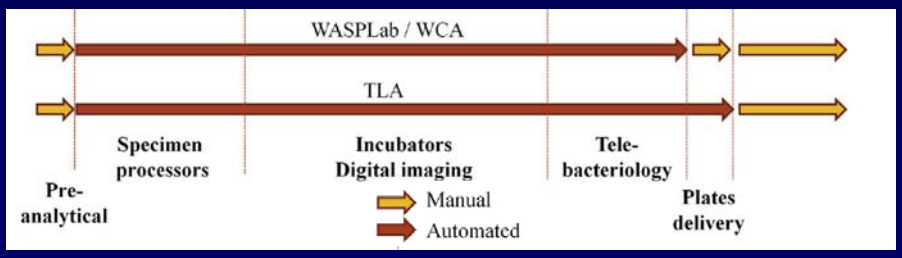


WASPLab (Copan)

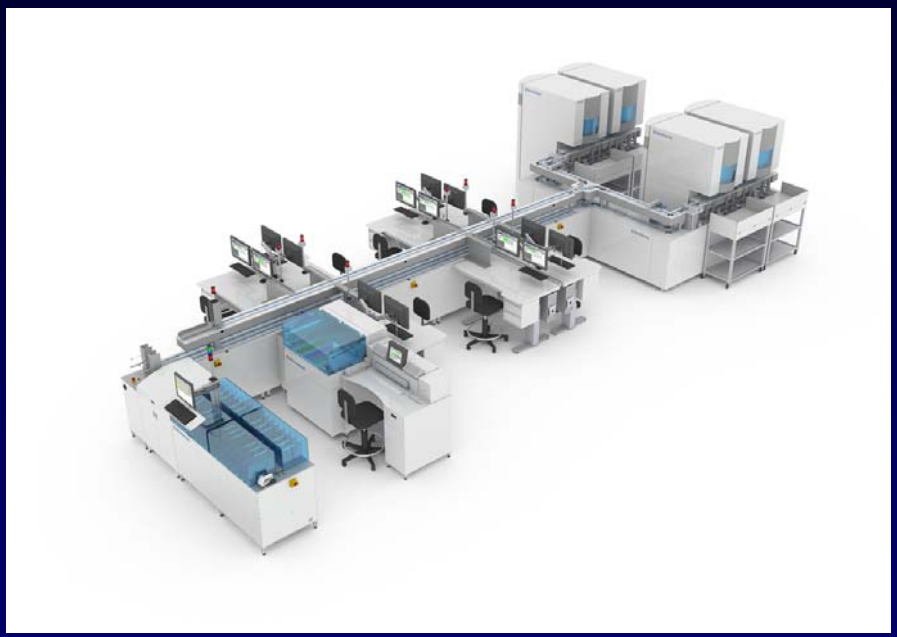
Work Cell Automation (WCA) (BD Kiestra)

Complete laboratory automation

Total Lab Automation (TLA) (BD Kiestra, The Netherlands)



Total Lab Automation (TLA) (BD Kiestra)



Incubators

constant and uniform T (laminar flow)

internal automated digital imaging system

camera	WASP	48 Mp
	Kiestra	5 Mp
size of image files	WASP	20–25 Mb
	Kiestra	3 Mb
light sources/background		front, back, side lights no or black background
capacity	WASP	882/1764 plates
	Kiestra	1152 plates

Telebacteriology

the use of digital imaging and file storage for

on-screen reading and decision making

Demonstrated advantages of laboratory automation (I)

specimen processors compared to manual streaking

- produce more isolated colonies
- exhibit enhanced reproducibility
- provide decreased hands-on plating time

J Clin Microbiol 2009;47:1101-6.
J Clin Microbiol 2015;53:2298-307.
J Clin Microbiol 2014;52:796-802.
J Clin Microbiol 2012;50:2732-6.

the higher yield of isolated colonies obtained with the InoqulA system compared to manual inoculation greatly decreased the requirement for subculturing and resulted in a significant decrease in time to result, laboratory workload and laboratory costs

J Clin Microbiol 2015;53:2298-307.

Demonstrated advantages of laboratory automation (II)

implementation of laboratory automation combined with MALDI-TOF allowed the TAT to significantly decrease for microbial identification of positive blood cultures, allowing adjustment of the antibiotic regimen in 12% of patients

Ann Lab Med 2014;34:111-7.

laboratory automation allowed a reduction of the TAT for urine specimens from 24 hours' to 16 hours' incubation, with a 99.7% clinical interpretation agreement

Bielli et al. ECCMID, 2015, abstract EVO535

Advantages of laboratory automation

true challenge remains to
assess the real clinical impact and benefits
that may be obtained
from faster test results and
improved laboratory efficiency

Clin Microbiol Infect 2016;22:217-35

Disadvantages of laboratory automation

Disadvantages
No laboratory adaptation to automation (e.g. staff shifts, training, 24/7)

- Misuse of tools
- Expectations for increased productivity not achieved

Crash of automat (backup needed).

- Good support and maintenance essential.
- Expensive maintenance budget.

Staff turnover (boring and lonely work?).

- Lab automation needs to be a project that includes everybody.
- Aim is not to replace experienced laboratory technicians but to assist them in their daily tasks.

Only eye is used.

- Smelling or other sensing of colony consistency disappears.
- More difficult to identify unusual/new species.

Security.

- Inoculation of sensitive samples (e.g. sputum, blood culture).
- Contamination of specimen processors and incubators (e.g. fungus spores, biosafety class 3 microorganisms).

Loss of microbiologic knowledge.

- Decrease in analytical variability.
- Standardized microbiologic factory (you find what you are looking for).

Clin Microbiol Infect 2016;22:217-35

Future developments

- automated colony-picking modules (for ID by MALDI-TOF and suspension preparation)

- fully automated disk diffusion AST

- inteligent digital imaging (development of intelligent algorithms and expert systems with different future applications)
  - microbial growth detection and quantification
  - presumptive identification of species growing on chromogenic agar
  - automated recognition of sister colonies from chromogenic and nonchromogenic agar

Faron ML,et al.
Automated scoring of chromogenic media for detection of methicillin-resistant *Staphylococcus aureus* by use of WASPLab image analysis software.
J Clin Microbiol 2016; 54: 620-4.

Faron ML, et al.
Automatic digital analysis of chromogenic media for vancomycin-resistant-enterococcus screens using Copan WASPLab.
J Clin Microbiol 2016; 54: 2464-9.

Kirn TJ. (editorial)
Automatic digital plate reading for surveillance cultures.
J Clin Microbiol 2016; 54: 2424-6.

automated digital analysis is highly sensitive
(no positive screening specimens were missed)

ensuring that plates with negative results could reliably be automatically read and reported by the system
to reduce the time and cost required for laboratories to perform large-volume screens

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale della Romagna

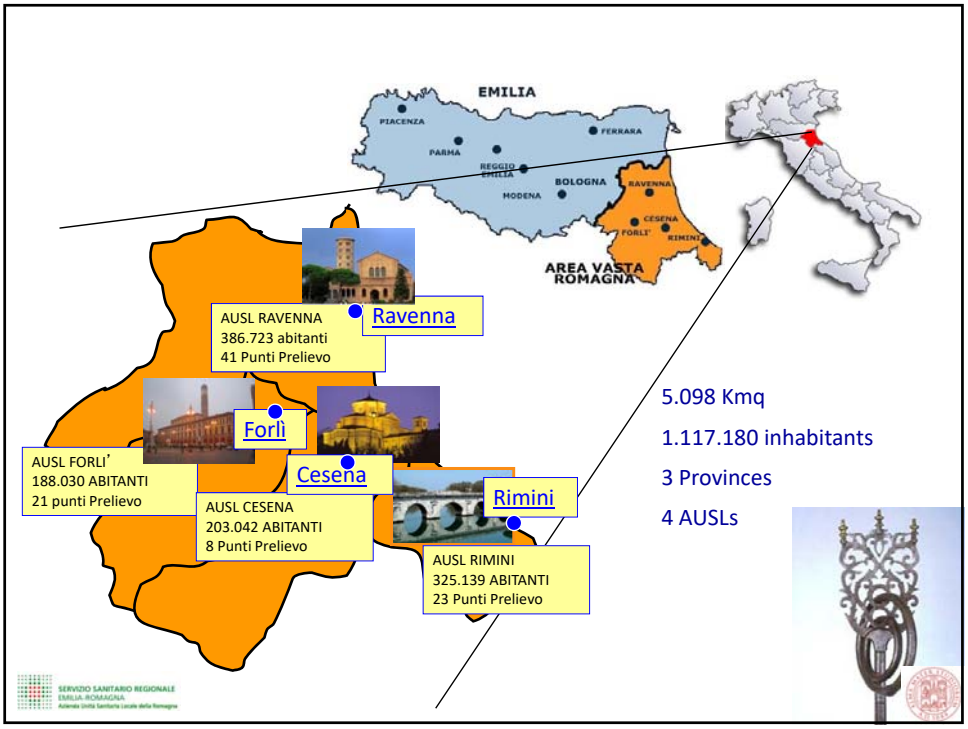
Consolidation of hospital laboratories – the experience of the Romagna Local Health Authority

Vittorio Sambri MD, PhD
Unit of Microbiology
The Great Romagna Hub Laboratory
Pievesestina, Cesena (Italy)
DIMES – University of Bologna (Italy)
vittorio.sambri@auslromagna.it – vittorio.sambri@unibo.it

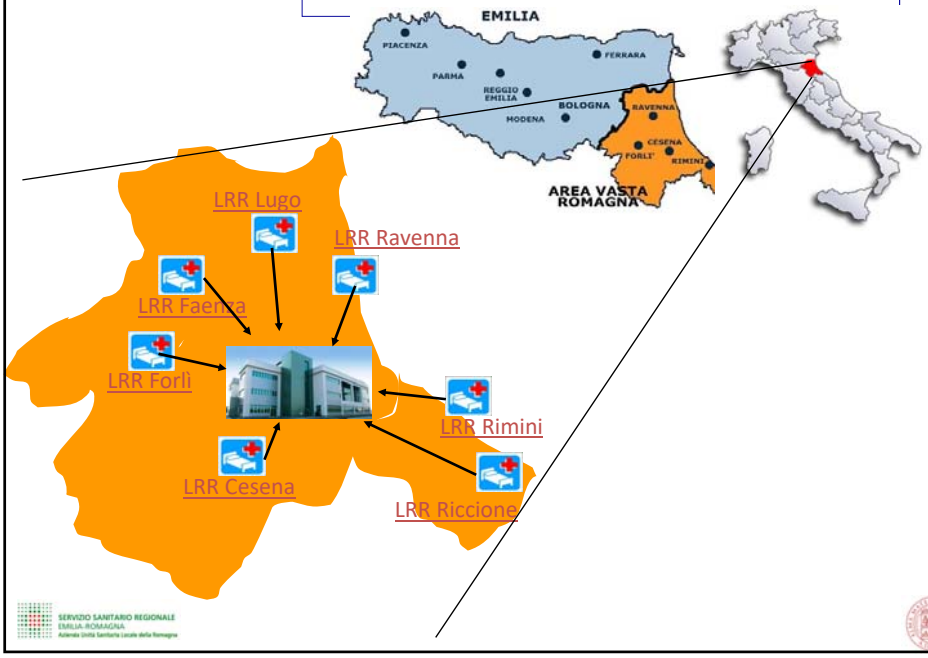
Overview

- driving forces for Consolidation
- The HUB LAB PVS (AUSL Romagna)
- Benefits & Drawbacks
- Future challenges
- Take Home messages

The Local Health Care Authority -AUSL



The AUSL Romagna HUB Laboratory -PVS



Consolidation…WHY?

To guarantee an integrated and modern Laboratory Medicine System that MUST be financially sustainable and totally integrated inside the Regional Health Care PUBLIC SYSTEM….

- **To Increase quality**
  - **To improve technology**
- **Total Financial sustainability**

Driving forces toward new and changing attitudes 2

Consolidation of laboratories

- The process is rapidly increasing in the US and EU, particularly for microbiology testing
- Larger laboratories have a greater potential to benefit from lab automation than smaller ones
- The 24-h, 24/7 microbiology laboratory is becoming common, and automation that can shorten turnaround time is required

Driving forces toward new and changing attitudes 1

Liquid-based microbiology.

- A paradigm shift occurred with the introduction of liquid based swab transport devices, first with **Eswab**
- the specimen is associated not with the swab but with the liquid phase of the transport device.
- liquid-based transport enables inoculation of the specimen and smear preparation with automated liquid-based specimen processors.

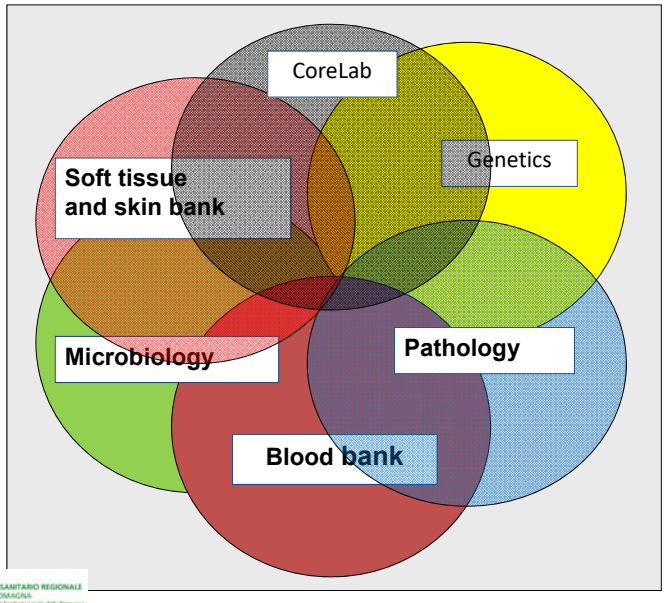
Driving forces toward new and changing attitudes 2

Personnel shortages

- Financial restrictions
  - ✓ Unit of Microbiology 2009: 82 (16 doctors/66 technicians)
  - ✓ Unit of Microbiology 2014: 53 (11 doctors/42 technicians)
- Retirement replacement ratio (currently is less than 25% in Italy)
- Technicians (and doctors) working in the (Microbiology) Lab are less paid than other health care professional

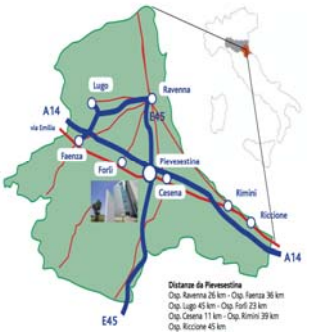
La.U.Ro. HUB LAB - PVS

La.U.Ro. HUB LAB - PVS



The Great Romagna Area: organization of Hub Laboratory

- Central Service Laboratory was born in March 2009. This service is responsible for all diagnostics tests of Romagna Area.
- The "hub and spoke" model is as follows:
  - **7 Quick-response laboratories located in 7 Decentralized Hospitals** (open 24h/7 days)
  - **1 PVS Central Laboratory HUB** organized in 3 operating units:
    - Clinical Pathology, **Microbiology** and Medical Genetics
  - 21 million tests/year (1.000.000 Microbiology)
  - Monday - Friday (8:00 to 18:30) Saturday – Sunday (8:00 to 16:00)



Roadmap toward the consolidation

1. 2002 planning begins
2. 2003 selection of the area for the new building
3. 2004 project begins
4. 2005 final project discussed with the workers UNIONS
5. 2007 the tenders start
6. 2008 infrastructure ready
7. 2009 operation begins

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA

Key POINTS along the pathway to consolidation

- Information Technology
- Technology
- People
- Building and Infrastructure
- Logistics

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EMILIA-ROMAGNA

People

	Before consolidation	After consolidation	2017
Physicians	34	19	12
Biologists	44	26	19
Technicians	245	240	192

More people working together on a larger number of patient (samples) with updated instruments and technologies.....

New Technologies

- Full automation for clinical pathology
- Full automation for serology
- Automation for Virology (Molecular)
- Automated pre-analitical steps in bacteriology

Infrastrucutres

All the Labs in the Hospitals required major renovation

	mq Laboratorio al 31.08.2008	mq LRR	Differenza mq
Cesena	**1.155**	**225**	**930**
Forlì	**1.150**	**200**	**950**
Faenza	**540**	**300**	**240**
Lugo	**640**	**400**	**240**
Ravenna	**780**	**450**	**320**
Riccione	**180**	**180**	**/**
Rimini	**1230**	**330**	**900**
Totale	**5.675**	**2.085**	**3.590**

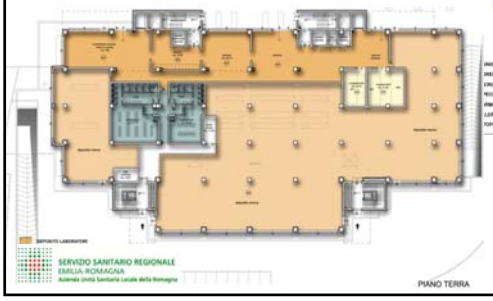
Consolidation made more than 3500 m² free space available in th eHospitalòs

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA

IL CENTRO SERVIZI DI AREA VASTA ROMAGNA

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA

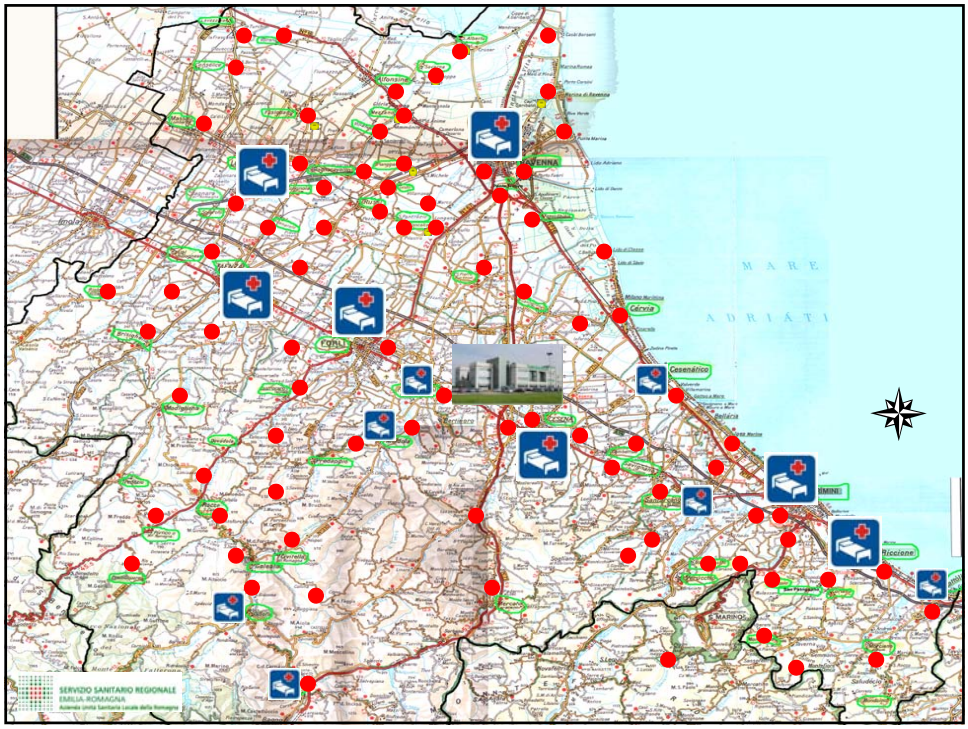
All the National and EU regulations for the diagnostic laboratories are FULLY IMPLEMENTED



The "Centro Servizi" includes 2 buildings

1) BUILDING "A": 10.500 m^2 /3 floors and includes all the diagnostics units

2) BUILDING "B": 8.700 m^2/ 2 floors and includes the General WAREHOUSE, the purchasing Unit and the General PHARMACY an



La strada dei campioni al laboratorio di microbiologia PVS

Corriere tragitto dei campioni la LRR Rimini verso il core lab:

- 8:30
- 10:00
- 12:00
- 13.30

Il viaggio dei campioni

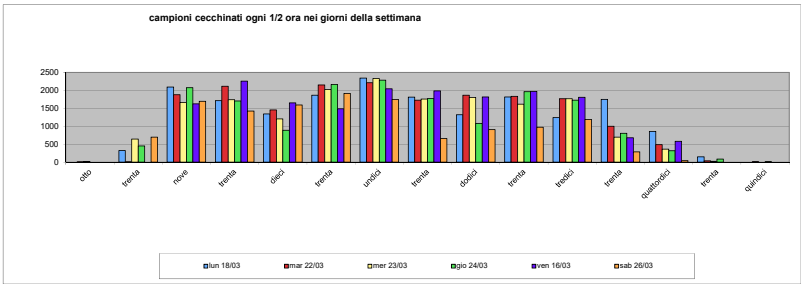
VAGINALI
URETRALI
ALTE E BASSE VIE RESP.
ALTRI MATERIALI FECALI

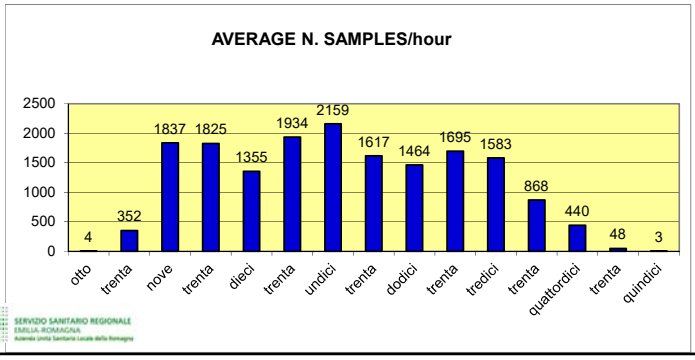
Dove viaggiano i nostri campioni?

- GPS positioning
- Truck license plate
- Starting Point
- Hour and date of starting
- Hour and date of arrival
- Temperature

SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA

Logistics

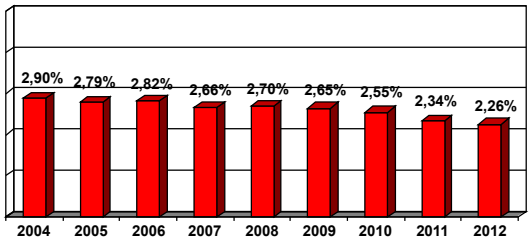




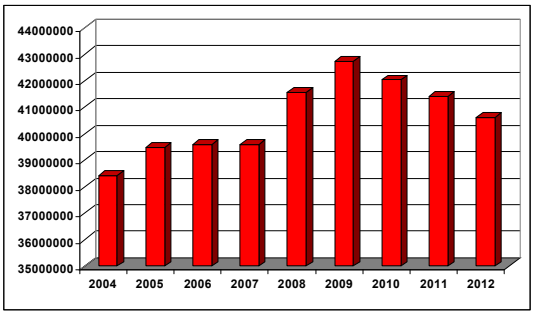
8	4
8.30	352
9	1837
9.30	1825
10	1355
10.30	1934
11	2159
11.30	1617
12	1464
12.30	1695
13	1583
13.30	868
14	440
14.30	48
15	3
tot	17182

Financial sustainability

Average cost of the Laboratory systems over the total Health Care expenses in the AUSL Romagna



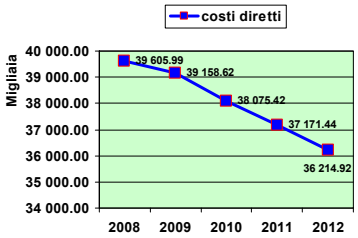
COSTS OF THE WHOLE LAB SYSTEM



2004	38 431 339
2005	39 471 948
2006	39 587 949
2007	39 607 577
2008	41 559 235
2009	42 733 580
2010	42 033 537
2011	41 416 685
2012	40 630 630

The direct costs include:

- personnel,
- instrumentations,
- maintenance,
- quality control,
- power..

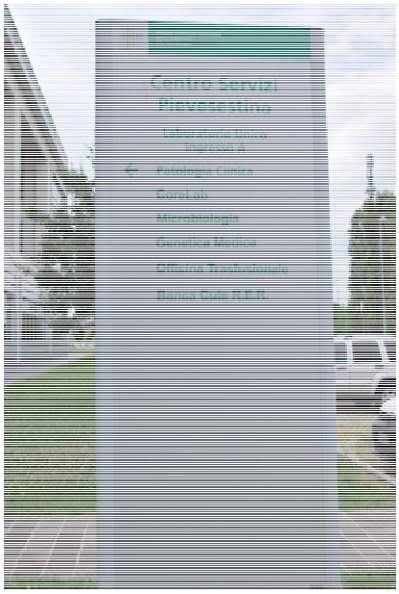


Historical impediments to automation

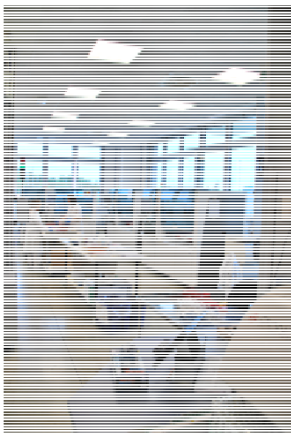
1. **Microbiology is too complex to automate.**
1. **No machine can replace a human in the microbiology laboratory.**
1. **Cost of automation.**
1. **Microbiology laboratories are too small for automation.**

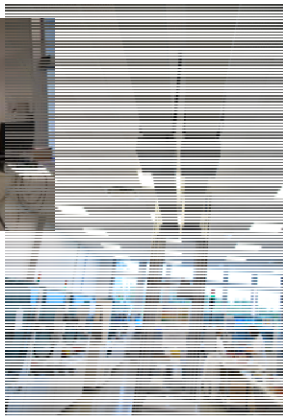
La.U.Ro. HUB Laboratory - PVS

Let’s start the tour



Where the samples arrive





Clinical Pathology

HPV DNA Screening

Molecular Virology

Serology

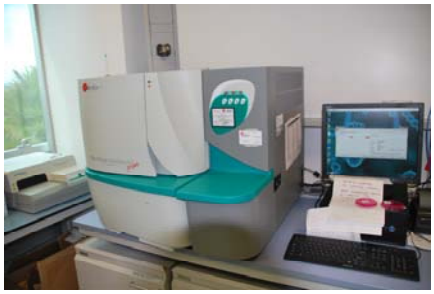
Serology

Laboratory Automation

Bacteriology

Serology - Virology


WASP 1
WASP

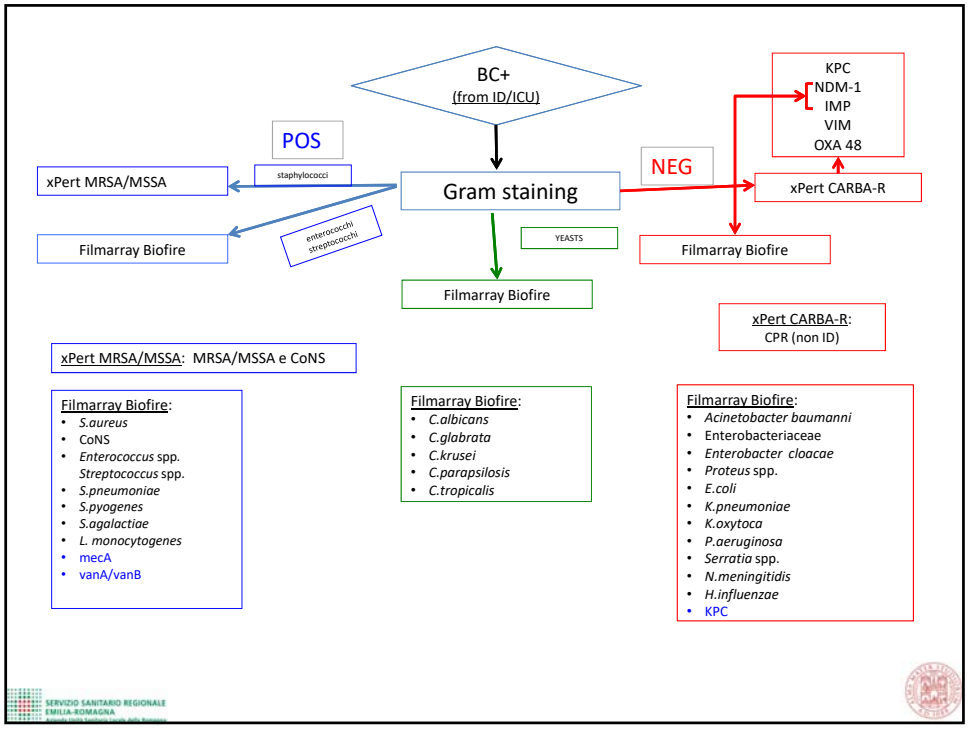


Still OPEN ISSUES

- **BLOOD CULTURES**
  - Incubation h 24/7 (spokes)
  - Only the POSITIVE are transferred to the HUB
  - Opening HOURS differ from HUB to SPOKE
  - **New regulatory rule for the accreditation of Microbiology Labs states that "evidence must be given that the BC workflow is not interrupted…."**

Still OPEN ISSUES….. Answers…

- BLOOD COLTURES
  - Implement more BC incubation slots in SPOKES
  - MALDI TOF
  - The "emo-FAST" algorithm
  - Hub open 7/7 – 12/24
  - Molecular (easy to use Film Array…) techniques in SPOKES



Risk-assessment may improve selection of patients with suspected sepsis for rapid diagnostics

Logan Ward[1,2], Michela Fantini[3], Vittorio Sambri[3], Steen Andreassen[1,2]

1. Treat Systems ApS, Aalborg, Denmark; 2. Aalborg University, Aalborg, Denmark; 3. Greater Romagna Area Hub Laboratory, Cesena, Italy

24 April 2017

27th ECCMID Vienna, Austria 22 – 25 April 2017

EMO FAST 3.0

SepsisFinder

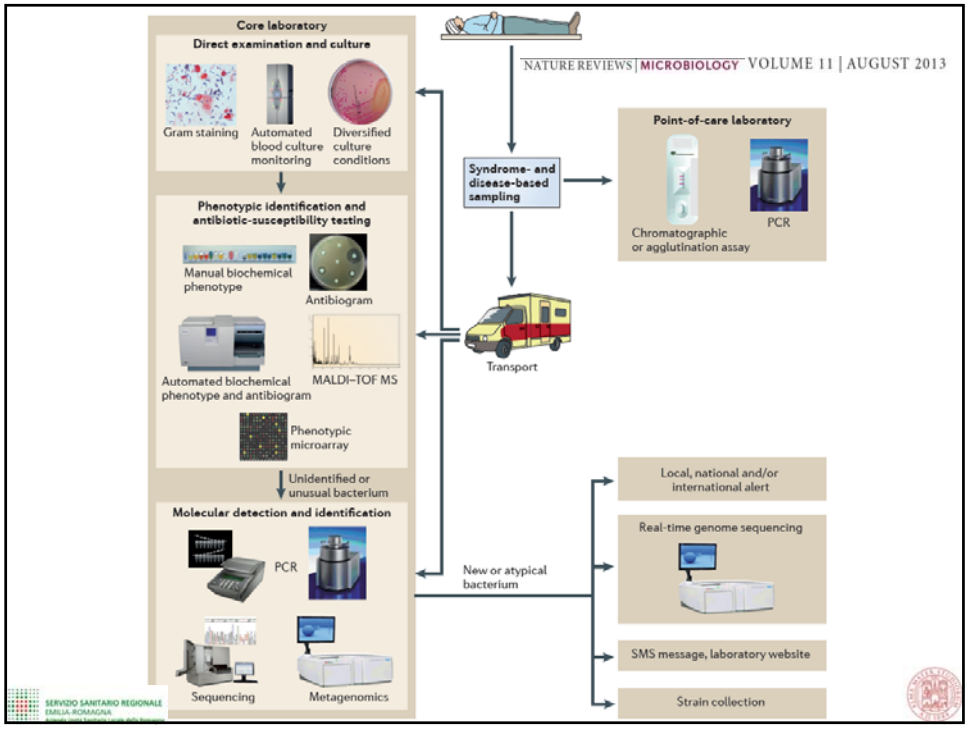
- Constant evaluation of the new technologies available
- Monthly re-evaluation of the workflow

  - Increase automation
  - Improve the clinical relevance of the Microbiology

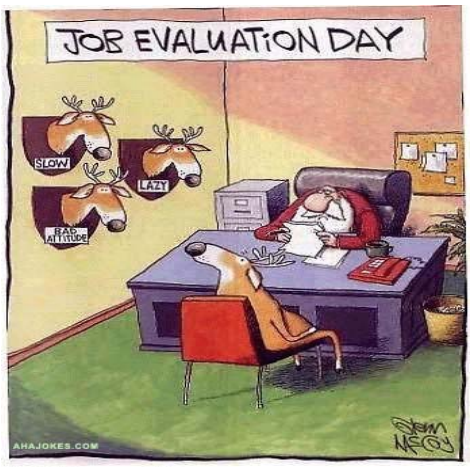
Driving forces toward new and changing attitudes: summary

To summarize the challenges currently being faced:

- microbiology laboratories are being asked to perform more testing (greater both in volume and complexity)
- to cope with increasing shortages of trained microbiology technologists
- to do all this in an economic climate where reimbursement is not likely to keep pace with the increasing costs.



Any serious consequence from the Full Automation……..?



SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA

Future Microbiology

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Unità Sanitaria Locale della Romagna

Skrbna raba antibiotikov

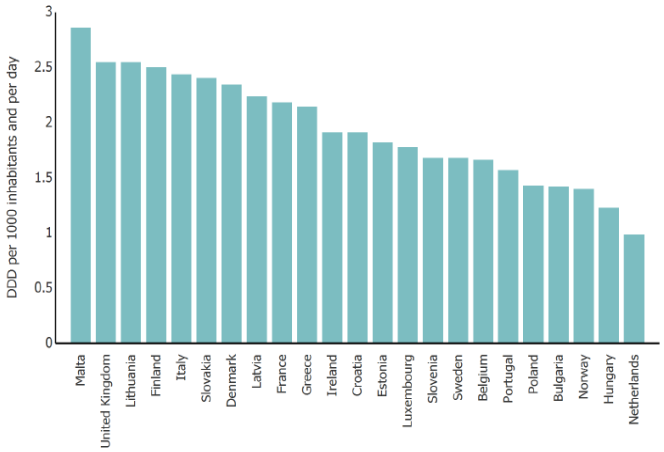
Bojana Beović

Univerza v Ljubljani
Medicinska fakulteta

univerzitetni
klinični center ljubljana
University Medical Centre Ljubljana

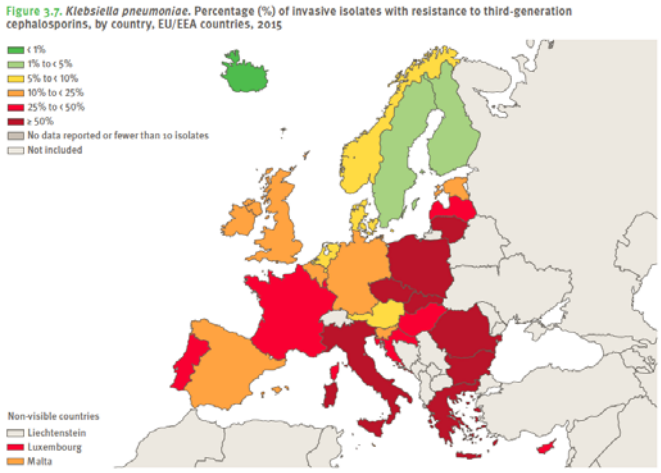
Velike razlike v predpisovanju antibiotikov v Evropi: posledica drugačne patologije?

Consumption of Antibacterials For Systemic Use (ATC group J01) in the hospital sector in Europe, reporting year 2015

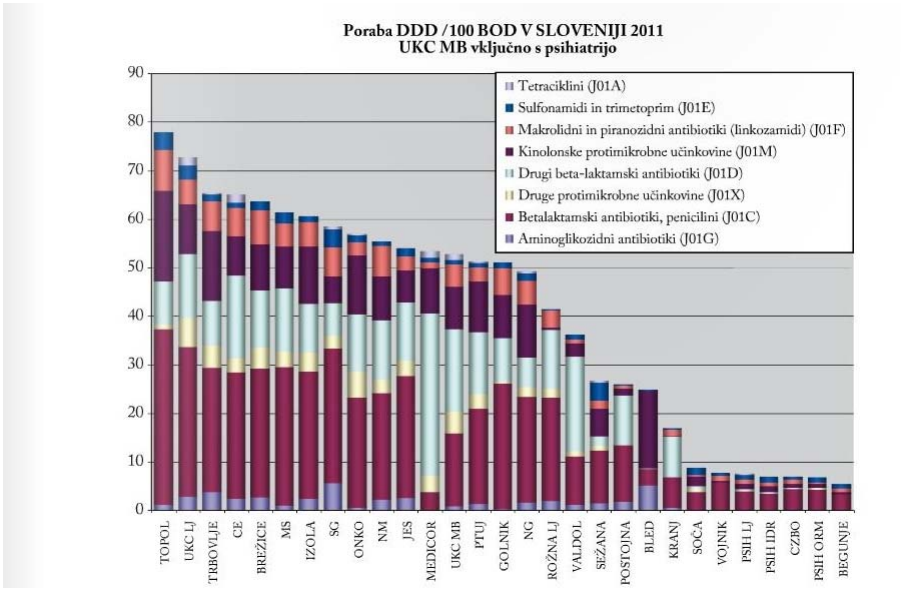


Adapted from www. http://ecdc.europa.eu/en/activities/surveillance/ESAC-Net/Pages/index.aspx

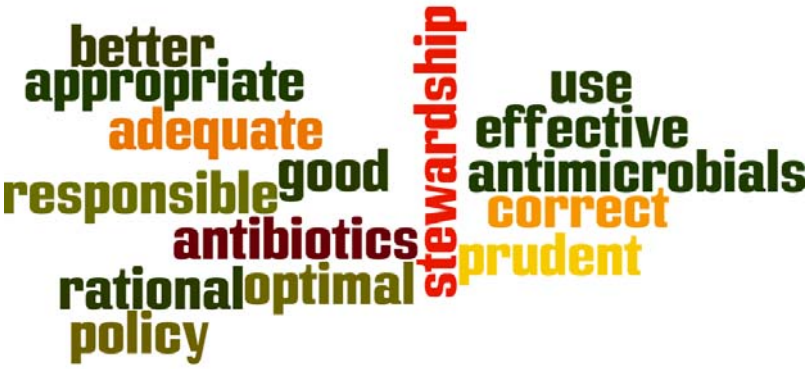
Klebsiella pneumoniae, odpornost proti 3.generaciji cefalosporinov



European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2017.



Čižman M, ISIS 2012



SUSTAINABLE USE OF ANTIMICROBIALS

Cilji nadzorovane rabe antibiotikov

Izboljšanje izidov zdravljenja vključno z manj neželenimi učinki.

Zmanjšanje protimikrobne odpornosti.

Optimizacija stroškov zdravljenja.

Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America Guidelines for Developing an Institutional Program to Enhance Antimicrobial Stewardship

Timothy H. Dellit,[1] Robert C. Owens,[2] John E. McGowan, Jr.,[3] Dale N. Gerding,[4] Robert A. Weinstein,[5] n,[6] Neil O. Fishman,[7] Christopher F. Carpenter,[10] P. J. Brennan,[9] Marianne Billeter,[11] and Thomas M. Hooton[12]

Clinical Infectious Diseases 2007; 44:159–77

…nadzorovana raba protimikrobnih zdravil je dejavnost, ki vključuje izbiro ustreznega protimikrobnega zdravila, odmerek, način odmerjanja in trajanje zdravljenja….

Clinical Infectious Diseases 2007; 44:159–77

INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY APRIL 2012, VOL. 33, NO. 4

SHEA/IDSA/PIDS POLICY STATEMENT

Policy Statement on Antimicrobial Stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS)

NADZOROVANA RABA PROTIMIKROBNIH ZDRAVIL

=

USKLAJENI UKREPI, KI IZBOLJŠAJO IN NADZORUJEJO PREDPISOVANJE PROTIMIKROBNIH ZDRAVIL

Pri nadzorovani rabi protimikrobnih zdravil gre za izvajanje smernic in drugih načel dobre klinične prakse.

Izvajanje načel nadzorovane rabe protimikrobnih zdravil mora doseči vse predpisovalce.

Sistematični (Cochrane) pregled intervencij za izboljšanje predpisovanja antibotikov v bolnišnicah
(vključene raziskave od 1980 do 2006)

- **89 raziskav, 95 intervencij**

- Učinek na predpisovanje antibiotikov:
  - ✓ Prepričevalne intervencije 3,5 do 42,3%
  - ✓ Restriktivne intervencije 17,1 do 40,5%
  - ✓ Strukturne spremembe 13,3 do 16,3%
- V raziskavah, ki so merile učinek na protimikrobno odpornost, se je zmanjšala odpornost proti različnim antibiotikom
- Povečanje ustreznega predpisovanja pri bolnikih s pljučnico je zmanjšalo smrtnost
- Raziskave, v katerih se je predpisovanje zmanjšalo, niso imele za posledico večje smrtnosti

Cochrane Database of Systematic Reviews 2013, Issue 4. Art. No.: CD003543.

Prepričevalni…

- Delitev izobraževalnih materialov
- Izobraževalni sestanki
- Lokalni usklajevalni sestanki
- Vizite s poučevanjem
- Lokalni mnenjski voditelji
- Opozorila verbalno, pisno ali v računalniku
- Nadzor s povratno informacijo

Davey P, Brown E, Charani E, Fenelon L,Gould IM,Holmes A, Ramsay CR,Wiffen PJ,WilcoxM. Interventions to improve antibiotic prescribing practices for hospital inpatients. Cochrane Database of Systematic Reviews 2013, Issue 4. Art. No.: CD003543.

Omejevalni ukrepi…

- Selektivno poročanje občutljivosti
- Omejen seznam zdravil v bolnišnici
- Obvezna odobritev določenih zdravil
- Avtomatična zamenjava zdravil
- Avtomatična zaustavitev zdravljenja
- Različne sheme zamenjav zdravil

Davey P, Brown E, Charani E, Fenelon L,Gould IM,Holmes A, Ramsay CR,Wiffen PJ,WilcoxM. Interventions to improve antibiotic prescribing practices for hospital inpatients. Cochrane Database of Systematic Reviews 2013, Issue 4. Art. No.: CD003543

Strukturni ukrepi

- **Zamenjava papirnatih popisov z elektronskimi vključno s predpisovanjem zdravil**
- **Hitre laboratorijske metode**
- **Informacijsko podprto odločanje**
- **Vpeljava mehanizmov za nadzor kakovosti**

Davey P, Brown E, Charani E, Fenelon L,Gould IM,Holmes A, Ramsay CR,Wiffen PJ,WilcoxM. Interventions to improve antibiotic prescribing practices for hospital inpatients. Cochrane Database of Systematic Reviews 2013, Issue 4. Art. No.: CD003543

Smernice za program smotrne rabe protimikrobnih zdravil v bolnišnicah IDSA&SHEA:
(močna priporočila, vse zmerna raven dokazov)

- **Preavtorizacija ali predpis zdravila po predhodnem posvetu in nasvetu (Pre-authorisation and/or prospective audit and feedback)**
- **Zmanjšanje predpisovanja antibiotikov, ki vplivajo na *Clostridium difficile***
- **Terapevtsko spremljanje koncentracij in prilagajanje odmerkov amonoglikozidov**
- **Peroralno antibiotično zdravljenje oziroma pravočasen preklop na peroralno zdravljenje**
- **Smernice in strategije za skrajšanje protimikrobnega zdravljenja na najkrajše učinkovito trajanje**

Tamar F, et al. Clin Infect Dis 2016.

German Society of Infectious Diseases: Antimicrobial Stewardship for Hospitals

- **Zahteve**
  - Delovna skupina za antibiotično nadzorstvo
  - Dostopnost podatkov o občutljivosti bakterij in porabi protimikrobnih zdravil
- **Ključne strategije**
  - Lokalne smernice, formularji protimikrobnih zdravil, omejitve predpisovanja, zahteve po odobritvi predpisa
  - Izobraževanje, usposabljanje, informacije
  - Proaktivni nadzor predpisovanja
  - Kazalniki kakovosti

De With K, et al. Infection 2016.

Ali so ukrepi nadzorovane rabe antibiotikov učinkoviti pri doseganju ciljev?

Ukrepi

- ✓ Izkustveno zdravljenje skladno s smernicami
- ✓ Deeskalacija
- ✓ Preklop z intravenskega na peroralno zdravljenje
- ✓ Spremljanje koncentracije zdravil
- ✓ Omejevanje uporabe nekaterih antibiotikov
- ✓ Konzultacije ob bolnikovi postelji

Cilji

- Klinični izidi
- Neželeni učinki
- Stroški
- Odpornost

Current evidence on hospital antimicrobial stewardship objectives: a systematic review and meta-analysis

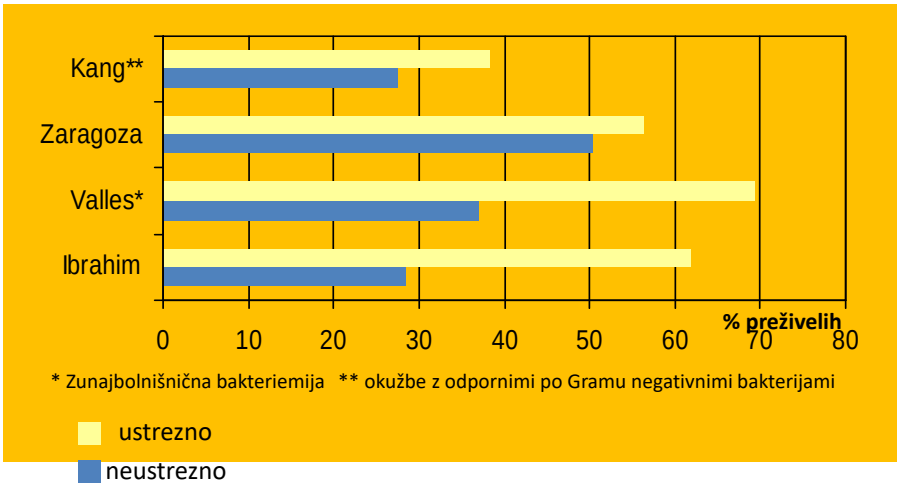
Emelie C Schuts, Marlies E J L Hulscher, Johan W Mouton, Cees M Verduin, James W T Cohen Stuart, Hans W P M Overdiek, Paul D van der Linden, Stephanie Natsch, Cees M P M Hertogh, Tom F W Wolfs, Jeroen A Schouten, Bart Jan Kullberg, Jan M Prins

Schuts EC, et al. Lancet Infect Dis 2016 Jul;16(7):847-56.

Vloga mikrobiološkega laboratorija pri skrbni (nadzorovani) rabi antibiotikov

- Podatki o lokalni občutljivosti bakterij za izbiro izkustvenega antibiotika
- Deeskalacija
- Hitra diagnostika za pravočasno izbiro ustreznega antibiotika
- Informacija za ukrepe za preprečevanje (prenosa) okužb

Pomen ustreznega izkustvenega zdravljenja za preživetje bolnikov z bakteriemijo



Ibrahim, et al. Chest 2000;118:146–155
Valles, et al. Chest 2003;123: 1615–1624
Zaragoza, et al. Clin Microbiol Infect 2003;9;412–418
Kang, et al. Antimicrob Agents Chemother 2005;49:760–766

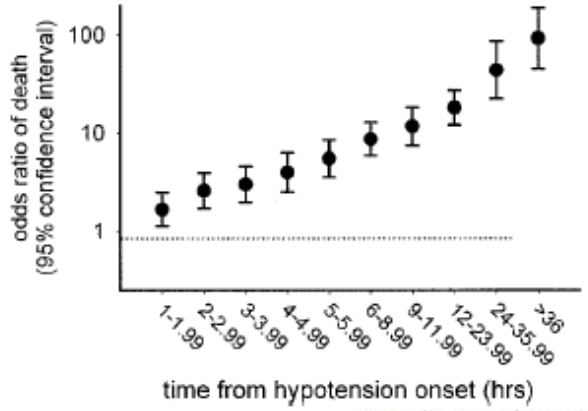
Deeskalacija je strategija, pri kateri zdravnik bodisi ukine antibiotik ali ga zamenja z ožjespektralnim antibiotikom po prejemu mikrobioloških izvidov.

Salahuddin N, et al. Critical Care Research and Practice Volume 2016, Article ID 6794861

Deeskalacija je strategija, pri kateri zdravnik bodisi ukine antibiotik ali ga zamenja z ožjespektralnim antibiotikom po prejemu mikrobioloških izvidov.

Salahuddin N, et al. Critical Care Research and Practice Volume 2016, Article ID 6794861

Povezanost tveganja za smrt in časa od začetka hipotenzije do ustrezne antibiotične terapija pri septičnem šoku



Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock*

Anand Kumar, MD; Daniel Roberts, MD; Kenneth E. Wood, DO; Bruce Light, MD; Joseph E. Parrillo, MD; Satendra Sharma, MD; Robert Suppes, BSc; Daniel Feinstein, MD; Sergio Zanotti, MD; Leo Taiberg, MD; David Gurka, MD; Aseem Kumar, PhD; Mary Cheang, MSc

Crit Care Med 2006; 34:1589–1596)

Klinični primer

27-letni bolnik

Operacija velikega meningeoma

Somnolenten (se izboljšuje), nepokreten

Koloniziran z ESBL, CRAb, CRPs

….postane febrilen 39°C, RR 110/50, fp 120/min, FD 28/min

	K. pneumoniae ESBL	CRPs	CRAb
ampicilin	R		
ampicilin/sulbaktam			NI (8)
amoks/klav	R		
pip/tazo	I	R	
cefuroksim	R		
cefotaksim	R		
ceftazidim	R	R	
cefepim	R	S	
imipenem	S	R (16)	R
meropenem	S	R (16)	
ertapenem	S		
gentamicin	R	S	R
amikacin	S	S	R
ciprofloksacin	R	S	R
levofloksacin	R	S	R
TMP/SMX	R		R
kolistin	S	S	S

Kateri antibiotik bi izbrali?

- Kolistin + amikacin
- Kolistin + meropenem
- Kolistin + ciprofloksacin + meropenem
- ….+ vankomicin (možnost okužbe CVK)

Kateri antibiotik bi izbrali?

- Kolistin
- Kolistin
- Kolistin m
- ….+ vankomicin (možnost okužbe CVK)

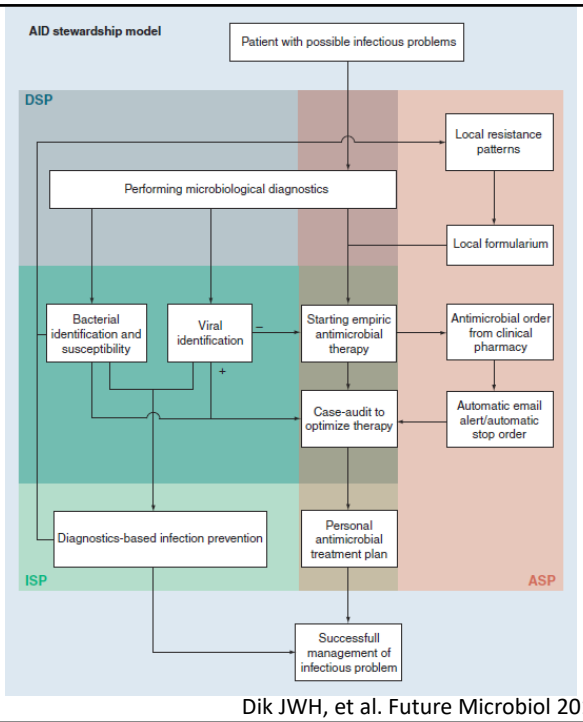
Integrirani model AID

Antimicrobial stewardship: nadzorovana (skrbna) raba protimikrobnih zdravil

Infection prevention stewardship: smotrno izbrani ukrepi za preprečevanje okužb

Diagnostic stewardship: nadzorovana (skrbna, smortna) raba diagnostičnih metod

Dik JWH, et al. Future Microbiol 2015; 11:93-102.



Dik JWH, et al. Future Microbiol 2015; 11:93-102.

Kombinacija hitre mikrobiološke diagnostike in ukrepov nadzorovane rabe antibiotikov

Review of Rapid Diagnostic Tests Used by Antimicrobial Stewardship Programs

Karri A. Bauer,[1] Katherine K. Perez,[2,3,4] Graeme N. Forrest,[5] and Debra A. Goff[1]

Pri večini raziskav je šlo za bakteriemijo, pri nekaterih za pljučnico ali okužbe mehkih tkiv.

Raziskave so vključevale bolnike s stafilokoknimi, enterokoknimi, po Gramu negativnimi in glivnimi (kandida) okužbami.

Nobena raziskava ni neposredno primerjala hitre metode z in brez sočasnega ukrepa nadzorovane rabe antibiotikov, a raziskave, pri katerih hitra diagnostična metoda ni imela vpliva na izboljšanje, niso vključevale ukrepov AS.

Clinical Infectious Diseases 2014;59(S3):S134–45

Review of Rapid Diagnostic Tests Used by Antimicrobial Stewardship Programs

Karri A. Bauer,[1] Katherine K. Perez,[2,3,4] Graeme N. Forrest,[5] and Debra A. Goff[1]

Rezultati raziskav, vključenih v pregled (17):

- Krajši čas do ustreznega zdravljenja
- Boljši izid zdravljenja
- Manjša smrtnost
- Manjša poraba določenih protimikrobnih zdravil
- Manjši stroški
- Krajše zdravljenje v bolnišnici

Clinical Infectious Diseases 2014;59(S3):S134–45

Vloga AS pri hitri mikrobiološki diagnostiki

Prava interpretacija	Ali zdravnik razume rezultat testa?*
Pravi antibiotik	Ali bo zdravnik izbral pravi antibiotik ob prejemu rezultata testa?
Pravi čas	Ali bo zdravnik dovolj hitro upošteval rezultat testa?

*Primeri vprašanj:

Povzročitelj?
Kolonizacija?
Izločanje po preboleli okužbi?
Senzitivnost testa (lažno negativen rezultat)?

Messacar K, et al. J Clin Microbiol 2017; 55: 715-23.

Table 3 Antimicrobial Stewardship Program Checklist for Rapid Diagnostic Tests

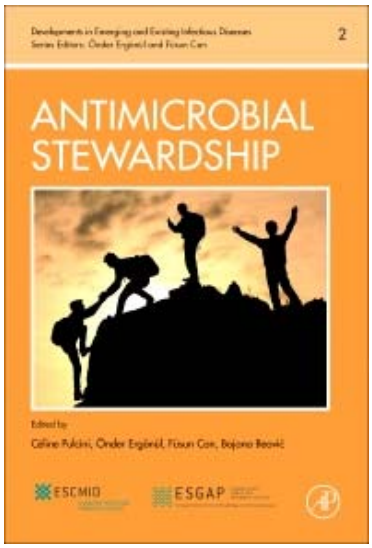
Preimplementation
- Identify most useful RDT based on hospital pathogen prevalence
  - Example: Number of *Staphylococcus aureus* bacteremias, number of coagulase-negative staphylococci, number of *Pseudomonas aeruginosa*, number of *Candida* species
- Identify hospital cost of infection
  - Example:
    - Utilize information warehouse personnel to pull cost by *ICD-9* code mortality data
    - Obtain time to ID specialist consult
    - Length of stay
    - 30-day readmission
- Time to effective therapy

Implementation
- Microbiologist-validated RDT instrument
- Determine if test is done in real time 24/7 or batch
- Communication of RDT results from microbiologist to physician and ASP pharmacist is established
- ASP pharmacist-physician educates medical staff
- ASP documents interventions and acceptance rate

Postimplementation
- Time to effective therapy
- Time to discontinuation or de-escalation
- Time to ID consult
- Documented negative blood culture prior to hospital discharge
- 30-day readmission
- Mortality

Clinical Infectious Diseases 2014;59(S3):S134–45

Hvala za pozornost!



Sindromska diagnostika

Miroslav Petrovec
skupaj z
Mateja Pirš, Mateja Poljšak Prijatelj, Miha Skvarč,
Andrej Steyer, Tjaša Cerar Kišek, Monika Jevšnik Virant, Tina Uršič
Inštitut za mikrobioogijo in imunolgijo
Medicinska fakulteta v Ljubljani
Zaloška 4,1000 Ljubljana

Sindrom - definicija

- **Sindrom** (grško σύνδρομο συν~, syn~: skupaj, z/s in δρόμος, drómos: pot, tek; torej kar poteka skupaj
- skupek vseh klinično izraženih bolezenskih znakov, simptomov, patoloških pojavov in značilnosti, ki se pojavljajo pri določeni bolezni

Sindromski pristop

- Namesto posamičnih tarč, s širokim pristopom **hkrati** iščemo nabor najverjetnejših mikroorganizmov, ki so lahko etiološko vpleteni v določen klinični sindrom

Pomembnejši sindromi

- Okužbe dihal – ILI – *influenza like illness*
- Okužbe prebavil/diareja
- Okužbe osrednjega živčevja
- Sepsa/bakteriemija
- Spolno prenosljive bolezni
- Okužbe oči, hepatitis, okužbe kosti…

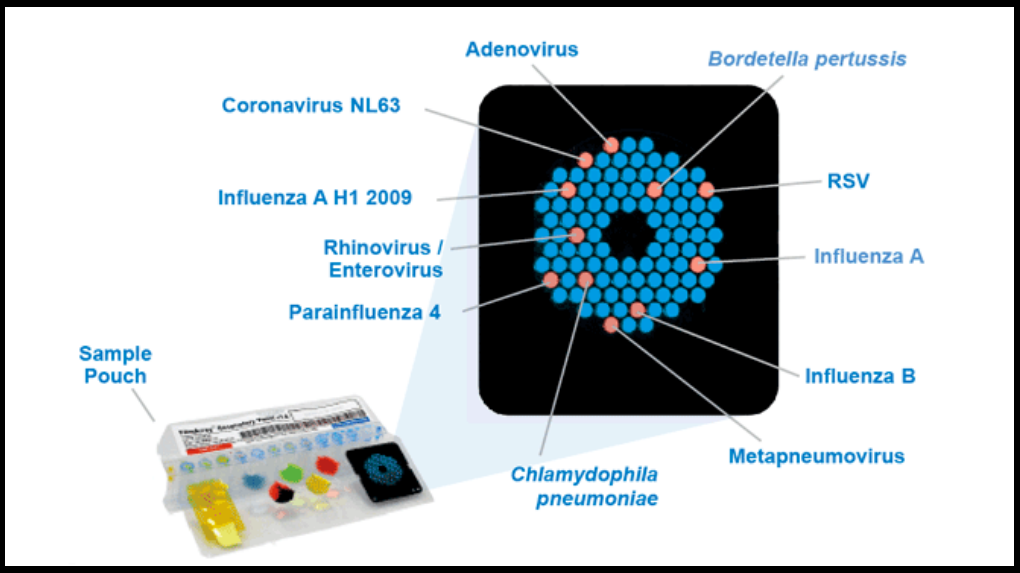
Laboratorijske platforme primerne za sindromski pristop k mikrobiološki diagnostiki

- **FilmArray**, Biofire - Biomerieux
- Iridica, Abott
- Luminex, Verigene, GenMark Diagnostics...
- Elitech, AusDiagnostics, Roche, Qiagen, Seegene...

FilmArray (Biofire, Biomerieux)

- Respiratorni panel (FDA, IVD)
  - 20 tarč, 17 virusov, 3 bakterije
- Gastrointestinalni panel (FDA, IVD)
  - 22 tarč, 14 bakterij, 4 paraziti, 4 virusi
- Panel za identifikacijo hemokultur, 27 tarč (FDA, IVD)
- ME panel
  - 16 tarč - 6 bakterij, 2 glivi, 8 virusov
  - Paneli v razvoju: tropske okužbe, bioterorizem ...

FilmArray 20 tarč (17 +3)



1 Test. 20 Respiratory Pathogens. All in about an hour.

Viruses

- Adenovirus
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus 229E
- Coronavirus OC43
- Human Metapneumovirus
- Human Rhinovirus/ Enterovirus
- Influenza A
- Influenza A/H1
- Influenza A/H1-2009
- Influenza A/H3
- Influenza B
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- Parainfluenza 4
- Respiratory Syncytial Virus

Bacteria

- *Bordetella pertussis*
- *Chlamydophila pneumoniae*
- *Mycoplasma pneumoniae*

Respiratorni panel - naše izkušnje

- V izvajanju od leta 2012
- Izredno hiter čas do rezultata – 85 minut, Zelo preprosta izvedba - potreben čas 5-7 minut
- Premajhna kapaciteta brez velikih investicij
- Manjkajoča tarča - bokavirus
- Visok odstotek neskladnih rezultatov
- Nedefinirane indikacije
- Visoka cena

Interpretacija - problemi

- Večkratno pozitivni vzorci - dihala, prebavila
- Arbitrarno vključevanje tarč - manjkajoče tarče (npr. bokavirusi)
- Neenakomerna občutljivost in specifičnost posameznih tarč (npr. *B. pertussis – prejšnje verzije testa*)
- Stroškovna učinkovitost in smiselnost uporabe v času epidemij
- Uporaba, kljub manjšemu naboru naročenih testov?
- Težavna komunikacija s kliniki - natančna opredelitev tarč
- Ponovljivost rezultatov

FilmArray®
Respiratory Panel IVD

FilmArray®
www.idahotech.com

Run Summary
Sample ID:
Bocavirus
Coronavirus NL63
Run Date: 12 Oct 2012
Controls: 1:46 PM
Passed

Večkratno pozitivni vzorci ???

Detected: *Mycoplasma pneumoniae*
Equivocal: None

Result Details

Result	Interpretation	Call	Assay
Not Detected	Adenovirus	Negative	Adeno
✓ Detected	Bocavirus	Positive	Boca
Not Detected	Coronavirus 229E	Negative	CoV-229E
Not Detected	Coronavirus HKU1	Negative	CoV-HKU1
✓ Detected	Coronavirus NL63	Positive	CoV-NL63
Not Detected	Coronavirus OC43	Negative	CoV-OC43
Not Detected	Human Metapneumovirus	Negative	hMPV
✓ Detected	Human Rhinovirus/Enterovirus	Negative	Entero1
		Negative	Entero2
		Positive	HRV1
		Positive	HRV2
		Positive	HRV3
		Positive	HRV4
Not Detected	Influenza A	Negative	FluA-H1-2009
		Negative	FluA-H1-pan
		Negative	FluA-H3
		Negative	FluA-pan1
		Negative	FluA-pan2
Not Detected	Influenza B	Negative	FluB
Not Detected	Parainfluenza Virus 1	Negative	PIV1
Not Detected	Parainfluenza Virus 2	Negative	PIV2
Not Detected	Parainfluenza Virus 3	Negative	PIV3
✓ Detected	Parainfluenza Virus 4	Positive	PIV4
✓ Detected	Respiratory Syncytial Virus	Positive	RSV
Not Detected	*Bordetella pertussis*	Negative	Bper
Not Detected	*Chlamydophila pneumoniae*	Negative	Cpne
✓ Detected	*Mycoplasma pneumoniae*	Positive	Mpne
Result	Control	Call	Assay
Pass	PCR2 Control	Positive	PCR2
Pass	RNA Process Control	Positive	yeasRNA

Manjkajoča tarča

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2011, p. 1179–1181 Vol. 49, No. 3
0095-1137/11/$12.00 doi:10.1128/JCM.02362-10

Human Bocavirus as the Cause of a Life-Threatening Infection∇

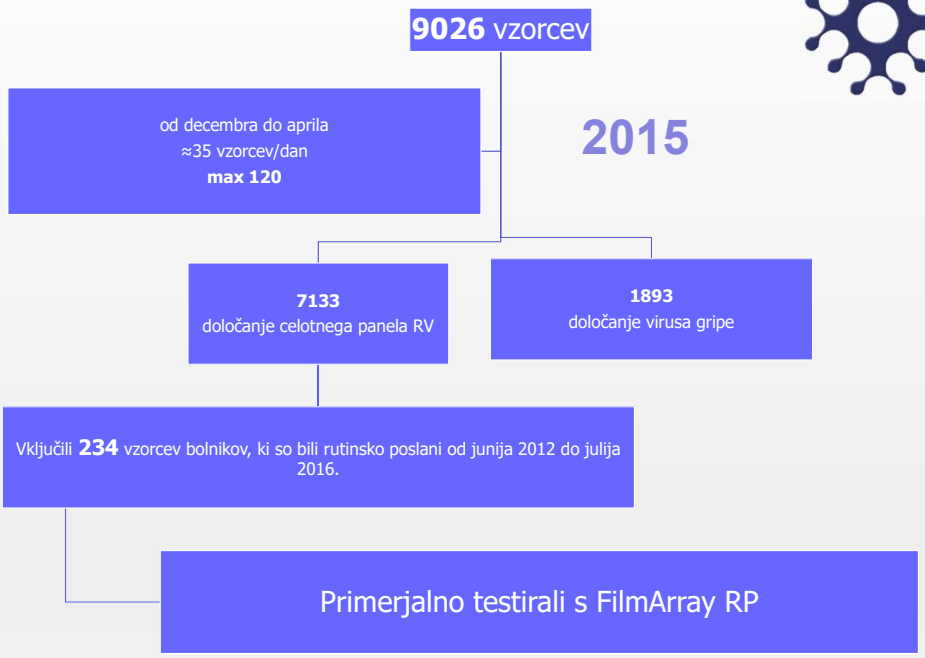
Tina Uršič,[1]* Andrej Steyer,[1] Silvester Kopriva,[2] Gorazd Kalan,[2] Uroš Krivec,[3] and Miroslav Petrovec[1]

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia[1]; Department of Pediatric Surgery and Intensive Care, University Medical Centre Ljubljana, Bohoričeva 20, 1000 Ljubljana, Slovenia[2]; and Department of Pulmonology, University Children's Hospital, University Medical Centre Ljubljana, Bohoričeva 20, 1000 Ljubljana, Slovenia[3]

FATAL HUMAN BOCAVIRUS INFECTION IN AN 18-MONTH-OLD CHILD WITH CHRONIC LUNG DISEASE OF PREMATURITY

Tina Uršič, BSc, PhD, Uroš Krivec, MD,† Gorazd Kalan, MD, MSc,‡ and Miroslav Petrovec, MD, PhD**

Respiratorni patogeni virusni panel vs Filmarray



FilmArray RP/ RT-PCR v realnem času

FilmArray RP

- 17 virusnih tarč + 3 bakterije

RT-PCR v realnem času

- 16 virusnih tarč

Pregled nabora tarčnih organizmov pri primerjalnih metodah RT-PCR v realnem času in FilmArray RP

FluA-H1-2009
FluA-H1-pan
FluA-H3
FluA-pan1
FluA-pan2

Organizem	Tarčni gen (RT-PCR)	RT-PCR	Tračni geni (Film Array RP)
RSV	polimeraza		matriks
HRV	5' UTR	hkratna	5' UTR
EAV	nukleokapsida		/
hMPV	nukleoprotein	hkratna	nukleoprotein
EHV1	glikoprotein		/
HCoV 229E	polimeraza 1b	hkratna	polimeraza
HCoV HKU1	polimeraza 1b		nukleoprotein
HCoV OC43	polimeraza 1b		nukleoprotein
HCoV NL63	polimeraza 1b		nukleoprotein
HBoV	NS1	hkratna	/
AdV	hekson		hekson
PIV1	polimeraza	hkratna	hemaglutinin
PIV2	polimeraza		fuzijski
PIV3	matriks		fuzijski
PIV4			fuzijski
Flu A	matriks	hkratna	matriks, NS1[4], HA1, HA3
Flu B	matriks		hemaglutinin
EV	5' UTR		5' UTR
B. pertussis	/		toksin
C. pneumoniae	/		ompA
M. pneumoniae	/		toksin

FilmArray RP/ RT-PCR v realnem času

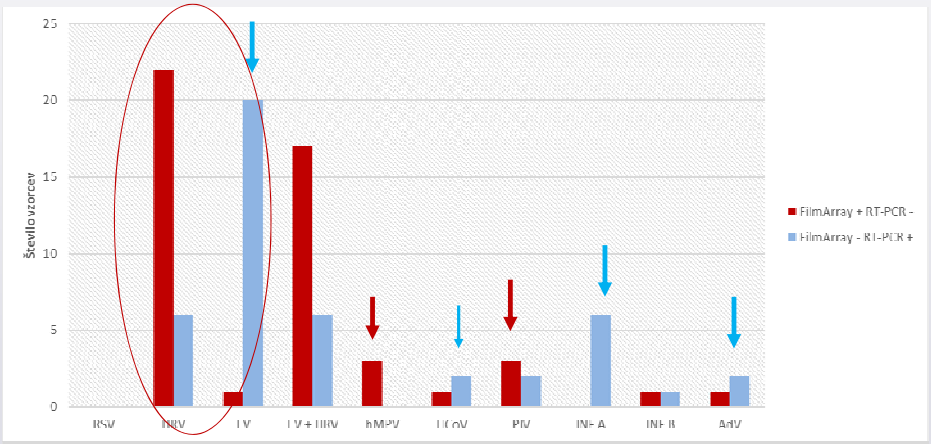
FilmArray RP

- 17 virusnih tarč + 3 bakterije
- Na enkrat lahko testiramo samo 1 pacienta.
- Izvid v 1 uri in 3 minutah.
- Enostavna za izvedbo.
- Ne potrebujemo visoko strokovno usposobljenega osebja.
- Nizka stopnja kontaminacije.

RT-PCR v realnem času

- 16 virusnih tarč
- Na enkrat lahko testiramo 10 pacientov (X 3)
- Izvid najkasneje v 3 urah od sprejema vzorca (x10)
- Zahtevna za izvedbo.
- Potrebujemo strokovno usposobljeno osebje
- Višja stopnja kontaminacije (potrebujemo primerno ločene prostore in instrumente)

Prikaz ujemanja metod FilmArray RP in RT-PCR v realnem času



FilmArray RP

Potrjena etiologija akutne okužbe dihal pri 135/234 (57,7 %) bolnikih.

RT-PCR v realnem času

Potrjena etiologija akutne okužbe dihal pri 122/234 (52,1 %) bolnikih.

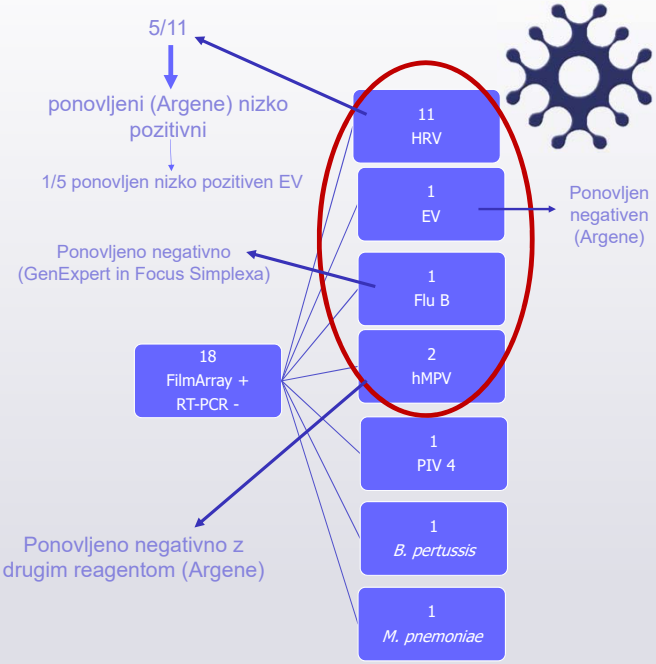


Table 1. Demographic and Clinical Information of Children With Pneumonia With No Identifiable Etiology and Control Subjects

	Patients (n = 70)	Control subjects (n = 90)	*P* value (χ^2)
Age group, no. (%)			.05
<1 y	14 (20)	23 (26)	
1 y	26 (37)	18 (20)	
2–4 y	30 (43)	49 (54)	
Month of enrollment, no. (%)			.04
January–March	23 (33)	13 (14)	
April–June	25 (36)	37 (41)	
July–September	16 (23)	25 (28)	
October–December	6 (9)	15 (17)	
Symptom, no. (%)			NA
Fever	67 (96)	NA	
Cough	58 (83)	NA	
Anorexia	53 (76)	NA	
Dyspnea	33 (47)	NA	
Underlying condition, no. (%)			ns
Asthma or reactive airway disease	6 (9)	3 (3)	
Preterm birth among children aged <2 y	7 (10)	7 (8)	
Radiographic findings, no. (%)			NA
Consolidation	32 (45)	NA	
Alveolar or interstitial infiltrate	23 (33)	NA	
Pleural effusion	20 (29)	NA	
Hospitalization			NA
Length of stay, median (IQR)	3 (2–4)	NA	
ICU admission, no. (%)	19 (27)	NA	
Death in the hospital, no. (%)	0 (0)	NA	

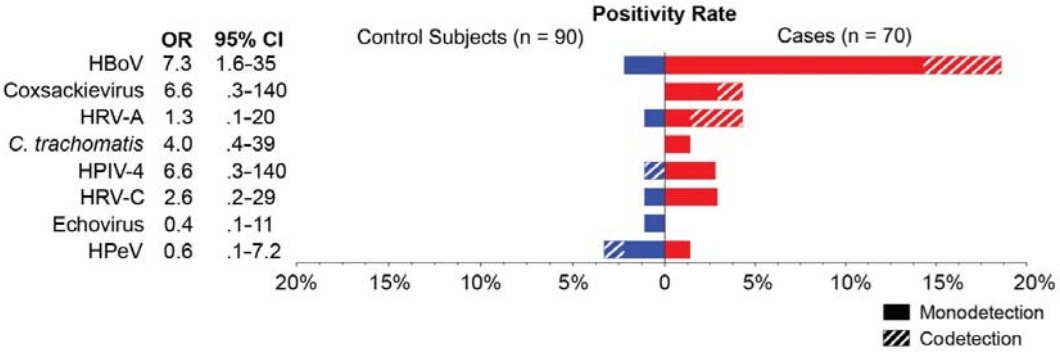
Abbreviations: ICU, intensive care unit; IQR, interquartile range; NA, not applicable; NS, not significant.

The Journal of Infectious Diseases
MAJOR ARTICLE

Viral Pathogen Detection by Metagenomics and Pan-Viral Group Polymerase Chain Reaction in Children With Pneumonia Lacking Identifiable Etiology

Robert Schlaberg,[1,2,a] Krista Queen,[4,a] Keith Simmon,[3] Keith Tardif,[3] Chris Stockmann,[3] Steven Flygare,[3] Brett Kennedy,[3] Karl Voelkerding,[1,3] Anna Bramley,[4] Jing Zhang,[4] Karen Eilbeck,[5] Mark Yandell,[6] Seema Jain,[4] Andrew T. Pavia,[7] Suxiang Tong,[4,b] and Krow Ampofo[3,b]

[1]Department of Pathology, [2]Department of Biomedical Informatics, [3]Department of Pediatrics, and [4]Department of Human Genetics, University of Utah, and [5]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, Utah; and [6]Centers for Disease Control and Prevention, Atlanta, Georgia



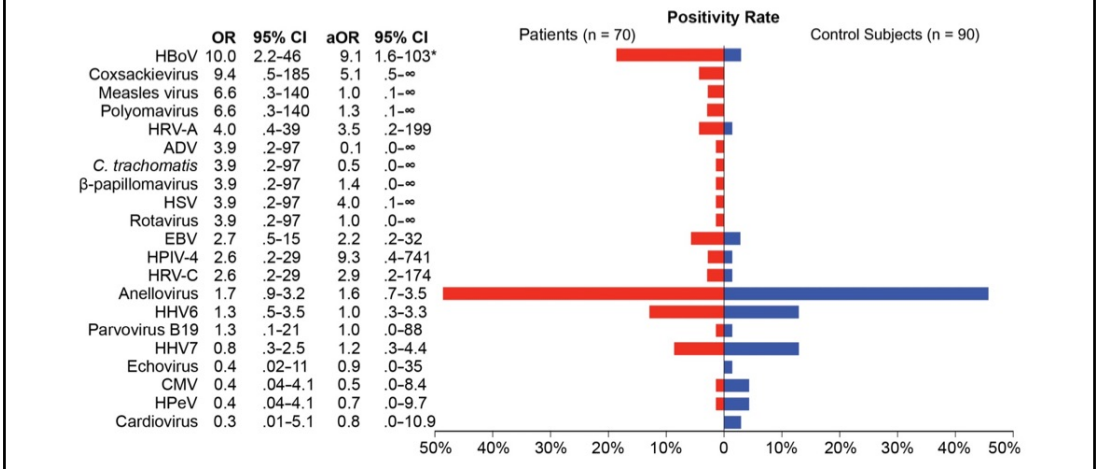
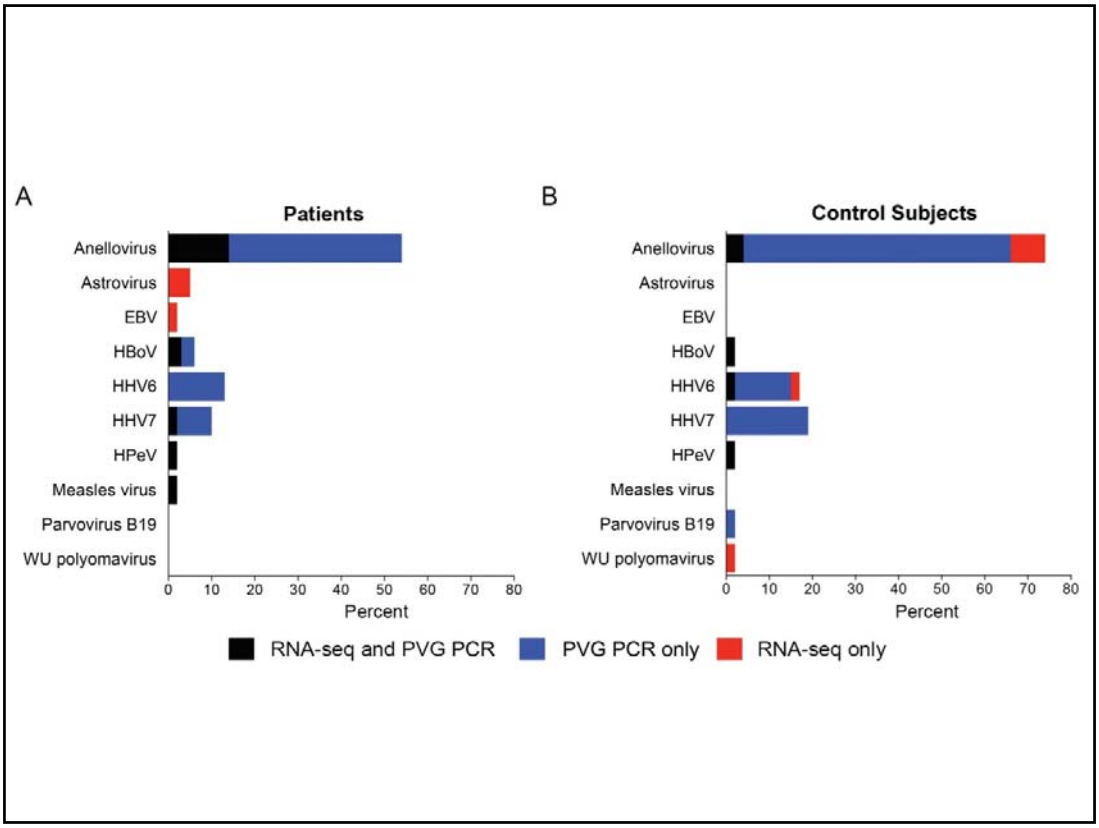


Figure 2. Viruses detected by RNA sequencing and/or pan-viral group polymerase chain reaction in children with pneumonia with no identifiable etiology (n = 70; red) and asymptomatic control subjects (n = 90; blue). A total of 20 different human viruses were detected in nasopharyngeal/oropharyngeal samples. In addition, *Chlamydia trachomatis* was detected in 1 newborn child with pneumonia. Fifteen viruses were more frequently detected in patients than control subjects (odds ratios >1), with human bocavirus ($P < .001$) having significant associations with community-acquired pneumonia. Abbreviations: ADV, adenovirus; aOR, adjusted odds ratio (adjusted for season and age group); *C. trachomatis*, *Chlamydia trachomatis*; CI, confidence interval; CMV, cytomegalovirus; HBoV, human bocavirus; HHV6, human herpesvirus 6; HHV7, human herpesvirus 7; HPeV, human parechovirus; HPIV-4, human parainfluenza virus type 4; HRV-A, human rhinovirus A; HRV-C, human rhinovirus C; HSV, herpes simplex virus; OR, odds ratio.

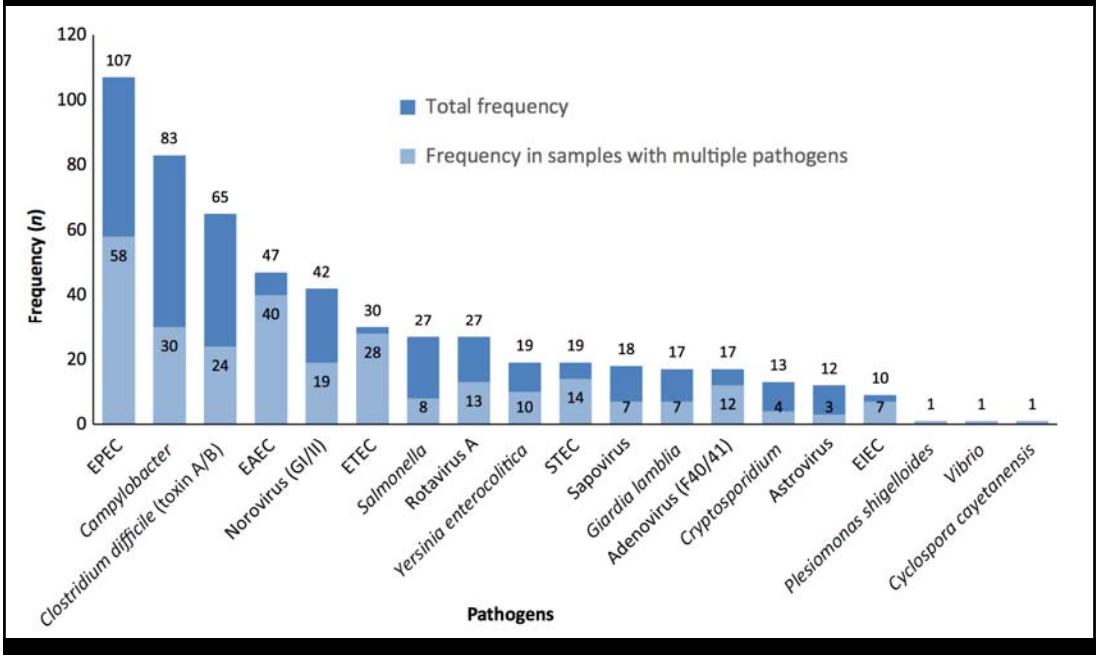


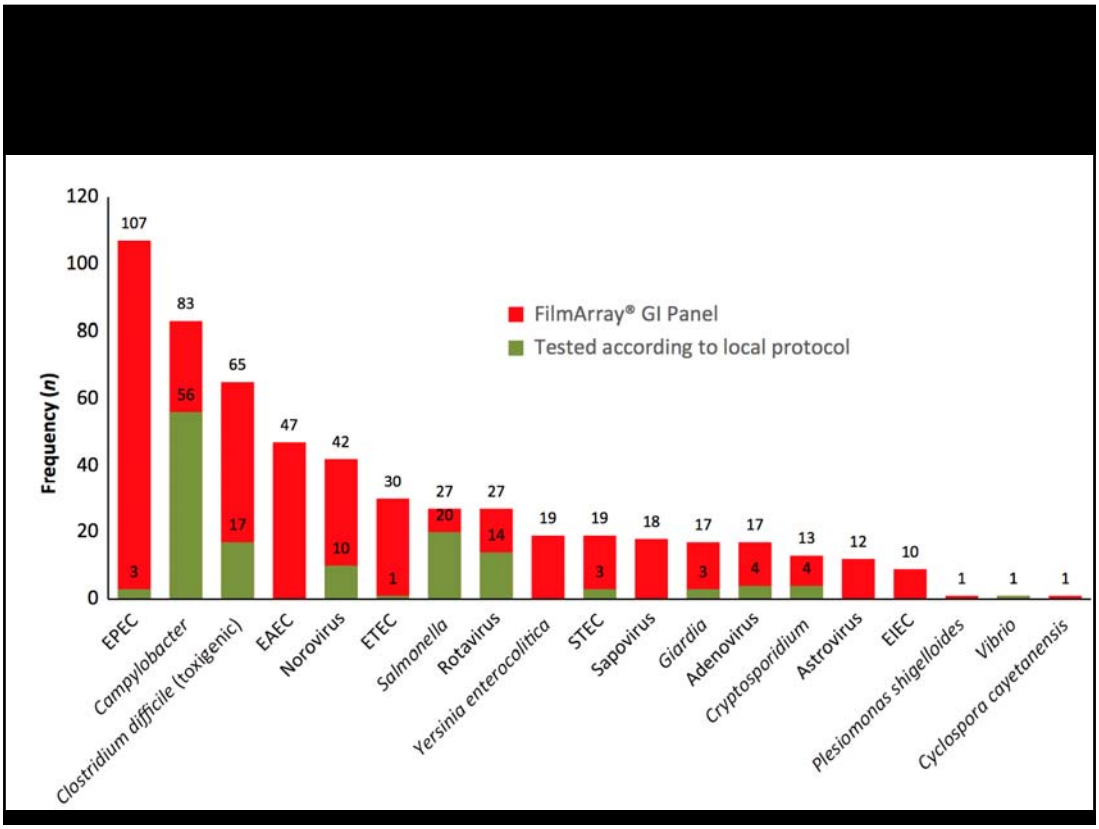
Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicentre study of community-acquired gastroenteritis

A. Spina[1], K. G. Kerr[2], M. Cormican[3], F. Barbut[4], A. Eigentler[5], L. Zerva[6], P. Tassios[6], G. A. Popescu[7], A. Rafila[7], E. Eerola[8], J. Batista[9], M. Maass[10], R. Aschbacher[11], K. E. P. Olsen[12] and F. Allerberger[1]

- Evropska multicentrična četrtletna študija EUCODI
- 4 določeni dnevi v letu 2014 – 20 zaporednih vzorcev, 10 laboratorijev iz 10 držav
- 709 vzorcev/bolnikov, 325 (45.8%) negativni, 268 (37.8%) en povzročitelj 116 (16.4%) več mikroorganizmov
- skupno pozitivnih 54,2 % testiranih vzorcev (384/709) vs 18,1% s konvencionalnimi metodami

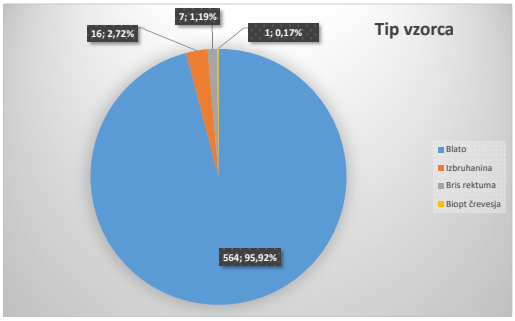
Clin Microbiol Infect 2015

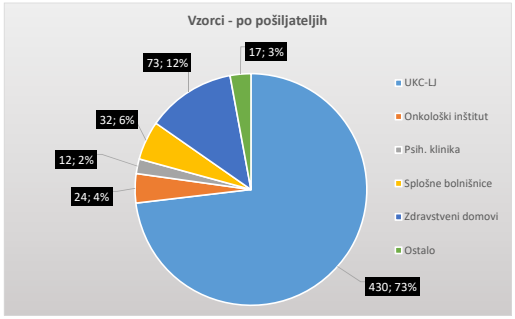




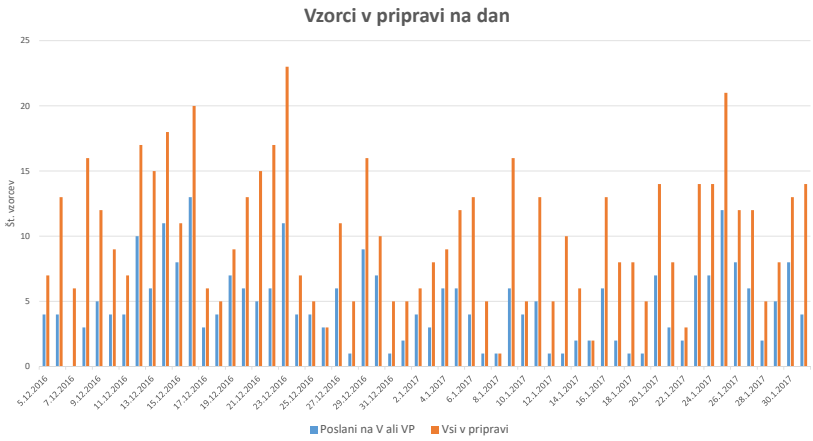
Vpeljava sindromske diagnostike za GE - IMI

VZORCI

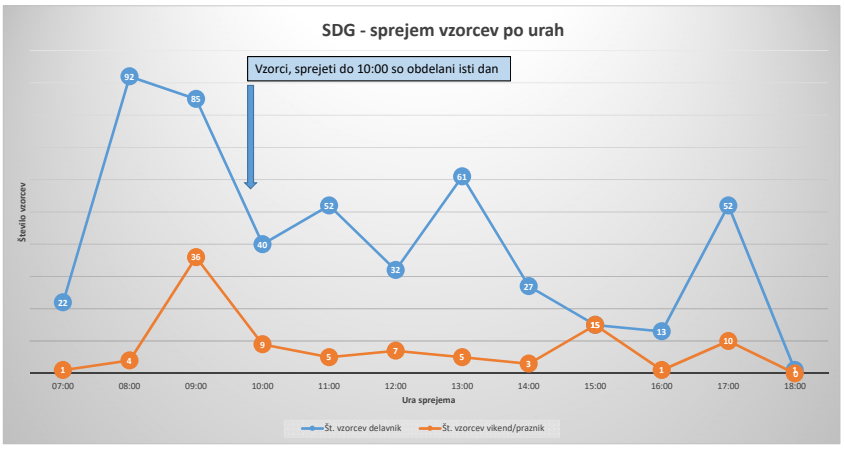




	Vzorci SDG (sezona 2016/17)	Vzorci V4 (sezona 2015/16)
December	300	305
Januar	288	255



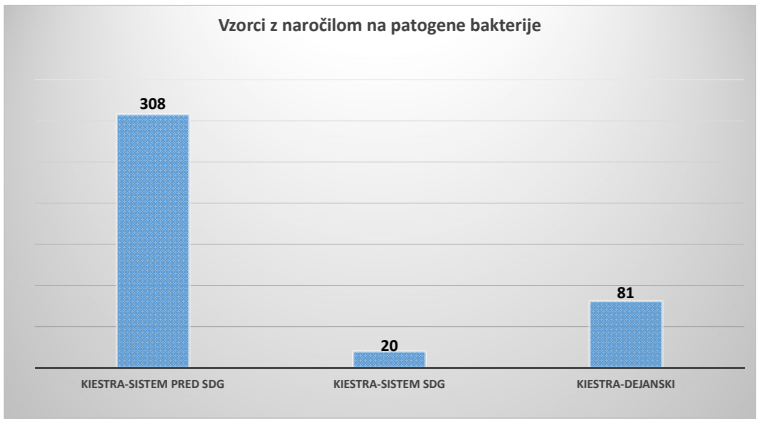
*z modrimi stolpci označeni vzorci, ki niso bili naročeni na patogene bakterije, oranžni stolpci predstavljajo vse vzorce, vpisane v SDG



Trenutno je med tednom in ob sobotah obdelano isti dan vse, kar je sprejeto do 10:00 (t.j. povprečno 40,8% dnevnih vzorcev)!

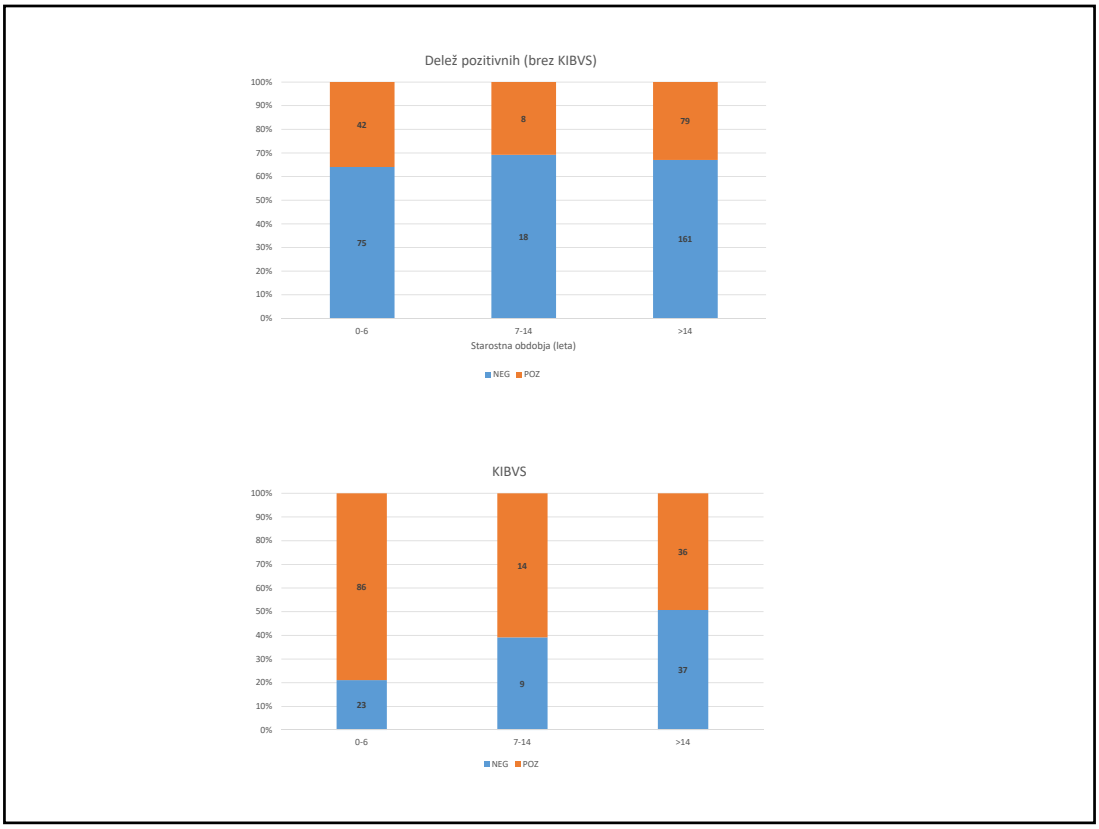
		DOKAZANE SKUPINE PATOGENOV							NEG	SKUPAJ
		V	B	P	V+B	V+P	B+P	V+B+P		
NAROČENO	V (n=256)	49,61	2,73	0,00	1,95	0,00	0,00	0,00	45,70	100
	BV (n=245)	28,57	4,90	0,41	1,22	0,82	0,00	0,41	63,67	100
	VP (n=5)	20,00	0,00	0,00	20,00	0,00	0,00	0,00	60,00	100
	BVP (n=43)	23,26	2,33	2,33	0,00	0,00	0,00	2,33	69,77	100
	SDG (n=20)	35,00	10,00	5,00	0,00	0,00	0,00	0,00	50,00	100
	P (n=2)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	100,00	100
	Posamezni (n= 17)	70,59	0,00	0,00	0,00	0,00	0,00	0,00	29,41	100
	SKUPAJ	38,61	3,74	0,51	1,53	0,34	0,00	0,34	54,93	100

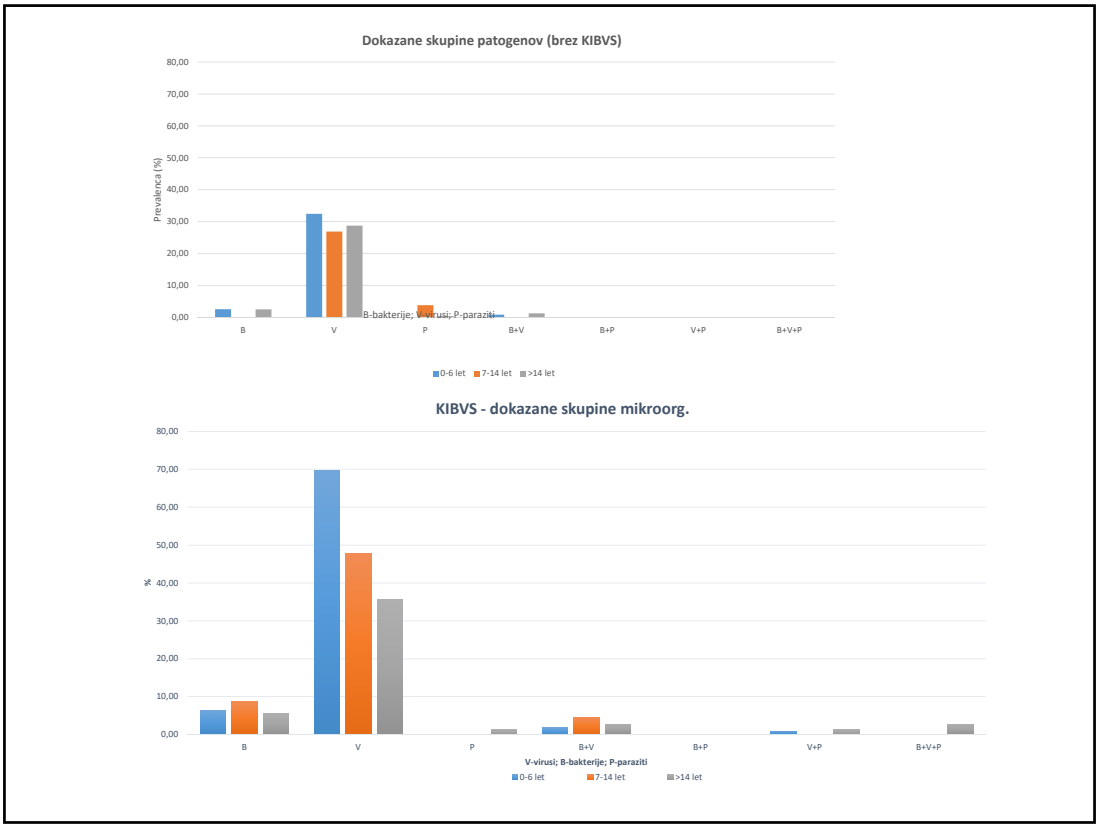
Le manjši delež vzorcev se nacepi na bakteriološki liniji…

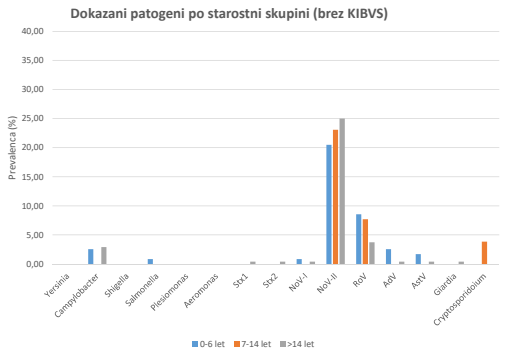


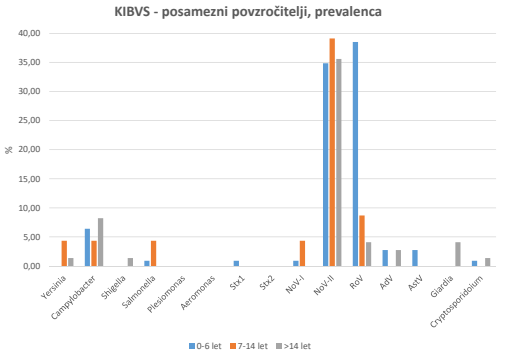
Le 6,5% vzorcev z naročilom na patogene bakterije bi se teoretično obdelalo na Kiestra-i (le molekularno pozitivni)

REALNO: 26,3 % vzorcev naročenih na patogene bakterije se je obdelalo na Kiestra-i (molekularno pozitivni in vzorci ob vikendih)


Delež pozitivnih (brez KIBVS)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
42
8
79
75
18
161
0-6
7-14
>14
Starostna obdobja (leta)
NEG
POZ
KIBVS
86
14
36
23
9
37
0-6
7-14
>14
NEG
POZ


Dokazane skupine patogenov (brez KIBVS)
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
Prevalenca (%)
B-bakterije; V-virusi; P-paraziti
B
V
P
B+V
B+P
V+P
B+V+P
0-6 let
7-14 let
>14 let
KIBVS - dokazane skupine mikroorg.
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
%
B
V
P
B+V
B+P
V+P
B+V+P
V-virusi; B-bakterije; P-paraziti
0-6 let
7-14 let
>14 let





bsolutnih številk:

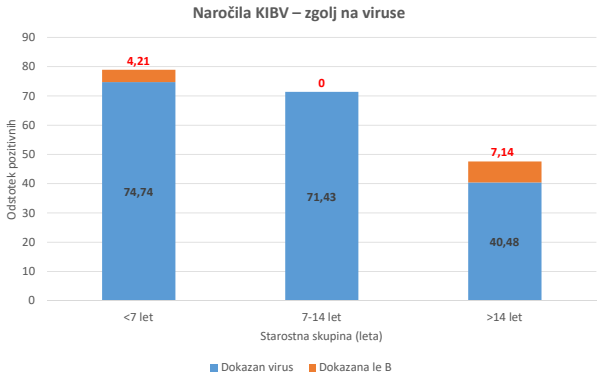
Zunanji	Vsi	NEG	B	V	P	B+V	B+P	V+P	B+V+P	POZ
0-6	117	75	3	38	0	1	0	0	0	42
7-14	26	18	0	7	1	0	0	0	0	8
>14	240	161	6	69	1	3	0	0	0	79
SKUPAJ	**383**	**254**	**9**	**114**	**2**	**4**	**0**	**0**	**0**	**129**
KIBVS	**Vsi**	**NEG**	**B**	**V**	**P**	**B+V**	**B+P**	**V+P**	**B+V+P**	**POZ**
0-6	109	23	7	76	0	2	0	1	0	86
7-14	23	9	2	11	0	1	0	0	0	14
>14	73	37	4	26	1	2	0	1	2	36
SKUPAJ	**205**	**69**	**13**	**113**	**1**	**5**	**0**	**2**	**2**	**136**

Zunanji	Vsi	Yersinia	Campylobacter	Shigella	Salmonella	Plesiomonas	Aeromonas	Stx1	Stx2
0-6	117	0	3	0	1	0	0	0	0
7-14	26	0	0	0	0	0	0	0	0
>14	240	0	7	0	0	0	0	1	1
SKUPAJ	**383**	**0**	**10**	**0**	**1**	**0**	**0**	**1**	**1**
KIBVS	**Vsi**	**Yersinia**	**Campylobacter**	**Shigella**	**Salmonella**	**Plesiomonas**	**Aeromonas**	**Stx1**	**Stx2**
0-6	109	0	7	0	1	0	0	1	0
7-14	23	1	1	0	1	0	0	0	0
>14	73	1	6	1	0	0	0	0	0
SKUPAJ	**205**	**2**	**14**	**1**	**2**	**0**	**0**	**1**	**0**

Zunanji	Vsi	NoV-I	NoV-II	RoV	AdV	AstV	Giardia	Cryptosporidoium
0-6	117	1	24	10	3	2	0	0
7-14	26	0	6	2	0	0	0	1
>14	240	1	60	9	1	1	1	0
KIBVS	**Vsi**	**NoV-I**	**NoV-II**	**RoV**	**AdV**	**AstV**	**Giardia**	**Cryptosporidoium**
0-6	109	1	38	42	3	3	0	1
7-14	23	1	9	2	0	0	0	0
>14	73	0	26	3	2	0	3	1
SKUPAJ	**205**	**2**	**73**	**47**	**5**	**3**	**3**	**2**

Dokazani molekularno – kultivacija neuspešna:

	prot-SD	Vrednost Ct	Ura sprejema	Dan sprejema
Yersinia	270/16	31,72	17:20	Sre
Campylobacter	55/16	30,93	17:10	Pet
	79/16	25,3	8:20	Pon
	29/17	28,7	8:25	Čet
	56/17	33,87	10:55	Sob
	60/17	33,37	7:55	Pon
	141/17	29,54	9:40	Sre
Stx1	73/16	34,62	8:20	Pon
	138/17	28,27	7:45	Sre



Pri vzorcih iz KIBV, pri katerih so naročili zgolj preiskavo na viruse, smo dodatno pojasnili manjši delež (do 7,14%) primerov AGE z bakterijsko etiologijo

Sklep

- Uporaba modernih molekularnih metod v sindromski obravnavi zahteva iskanje ravnotežja med tem kaj **potrebujemo**, kaj **zmoremo** in tem kaj si lahko **privoščimo**
- Laboratorij mora biti usmerjen na **vrednost** in **klinično uporabnost** in ne na **količino** testiranj (*Value - not volume driven*)

Novosti pri uporabi MALDI - TOF

asist. Julija Germ, dr. med.
doc. dr. Mateja Pirš, dr. med.

Likarjev simpozij 2017

Inštitut za mikrobiologijo in imunologijo, UL MF

MALDI - TOF in

-> rutinska identifikacija bakterijskih izolatov

-> neposredna identifikacija bakterij iz kliničnih vzorcev

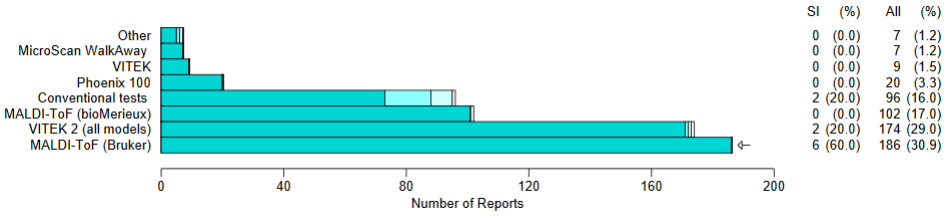
-> posledice uporabe MALDI-TOF za potek zdravljenja

-> določanje občutljivosti za antibiotike in detekcija mehanizmov odpornosti

-> tipizacija bakterij

UK NEQAS Microbiology

General bacteriology

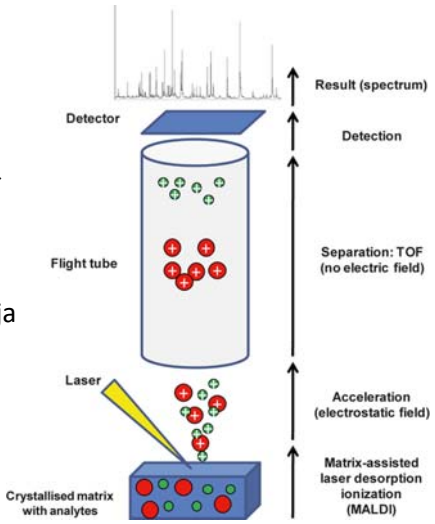


IDENTIFIKACIJA z MALDI - TOF

- **vsi laboratoriji: 47,9 %**
- **Slovenija: 60%**

MALDI-TOF: MATRIX-ASSISTED LASER DESORBTION IONIZATION – TIME OF FLIGHT

1. jekleni nosilec → vzorec → matriks
2. laser
3. uplinjenje vzorca + matriksa → oblak potuje skozi masni analizator do ionskega detektorja
4. analiza razmerja mase & naboja (2 - 20 kDA) – prevladujejo znotrajcelični hidrofilni strukturni ali ribosomalni proteini
5. masni spekter
6. knjižnica → identifikacija



Croxatto et al. FEMS Microbiol Rev 2012;36:380-407

Bruker
MBT v7311: 2509 vrst / 433 rodov
Mikobakterije: 164 vrst

bioMérieux V3 DB
Bakterije, kvasovke, plesni,
mikobakterije, Nocardia: 1046 vrst

RUTINSKA IDENTIFIKACIJA BAKTERIJSKIH IZOLATOV Z MALDI - TOF

JOURNAL OF CLINICAL MICROBIOLOGY, May 2010, p. 1549–1554 Vol. 48, No. 5
0095-1137/10/$12.00 doi:10.1128/JCM.01794-09

Performance of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of Bacterial Strains Routinely Isolated in a Clinical Microbiology Laboratory▽

A. Bizzini, C. Durussel, J. Bille, G. Greub,†* and G. Prod'hom†*

1371 izolatov

Direktna metoda / delna ekstrakcija z mravljično kislino (Bruker Daltonics) + primerjava s klasično identifikacijo

Identifikacija do vrste **93,2 %** -> vendar **4,9 %** diskordantnih rezultatov

Identifikacija do rodu **5,3 %**

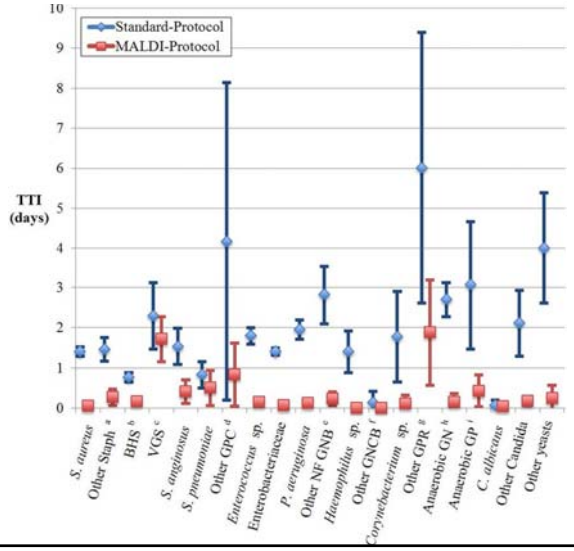
Brez identifikacije **1,5 %**

Prospective Evaluation of a Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System in a Hospital Clinical Microbiology Laboratory for Identification of Bacteria and Yeasts: a Bench-by-Bench Study for Assessing the Impact on Time to Identification and Cost-Effectiveness

K. E. Tan,[a] B. C. Ellis,[b] R. Lee,[b] P. D. Stamper,[a] S. X. Zhang,[a,b] and K. C. Carroll[a,b]

J Clin Microbiol 2012

Čas do identifikacije (TTI) in 95% interval zaupanja



Cost Savings Realized by Implementation of Routine Microbiological Identification by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Anthony Tran,[a]* Kevin Alby,[a]* Alan Kerr,[a] Melissa Jones,[a] Peter H. Gilligan[a,b]

TABLE 1 Reagent cost comparison between traditional and MALDI-TOF MS methods

Organism	No. of samples	Reagent costs ($)		Cost savings	
		Traditional	MALDI-TOF	$	%
Enterobacteriaceae	7,503	27,407.46	3,226.29	24,181.17	88.2
Enterococcus spp.	1,454	8,361.20	625.22	7,735.98	92.5
GNF[a]	3,489	21,154.03	1,501.56	19,652.47	92.9
Staphylococcus spp.	5,790	8,003.24	2,489.70	5,513.54	68.9
Streptococcus spp.	2,332	10,149.14	1,002.76	9,146.38	90.1
Yeast	1,362	3,614.55	735.48	2,879.07	79.7
Total	21,930	78,689.62	9,581.01	69,108.61	87.8

[a] Gram-negative glucose nonfermenters.

TABLE 3 Total cost comparison between traditional and MALDI-TOF MS methods, including maintenance agreement costs

Organism	No. of samples	Total cost ($)		Cost savings	
		Traditional	MALDI-TOF	$	%
Enterobacteriaceae	7,503	51,717.18	23,516.72	28,200.46	54.5
Enterococcus spp.	1,454	13,072.16	4,557.29	8,514.87	65.1
GNF[a]	3,489	32,458.39	10,936.89	21,521.50	66.3
Staphylococcus spp.	5,790	17,383.04	18,147.65	−764.61	−4.4
Streptococcus spp.	2,332	17,704.82	7,309.21	10,395.61	58.7
Yeast	1,362	10,197.09	4,418.75	5,778.34	56.7
Total	21,930	142,532.69	68,886.51	73,646.18	51.7

Ključni dejavniki za dobro ID do vrste

VPLIV NA KVALITETO SPEKTRA

starost kolonije

pogoji kultivacije

postopek ekstrakcije proteinov

ni pomemben dejavnik:
Escherichia coli in
Shigella spp

slabša kvaliteta spektra:
Streptococcus pneumoniae/
Streptococcus mitis

KVALITETA MALDI - TOF KNJIŽNICE

Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology

Antony Croxatto, Guy Prod'hom & Gilbert Greub

Table 2. Problems commonly found in routine identification by MALDI-TOF MS

Problems	Examples
Limit of resolution of the MALDI-TOF MS method	*Shigella* spp. identified as *E. coli*
Database discordances	*Propionibacterium acnes* wrongly identified as *Eubacterium brachy*
Errors in the reference spectra	due to incorrect reference spectra in the database
Similarities of spectra present in the database*	Incomplete reference librairies for viridans streptococci and pneumococci
Absence or insufficient reference spectra in the database*	No reference of non-*Clostridium* anaerobes in the database
	Insufficient number of reference spectra of *Streptococcus pneumoniae* and *Streptococcus parasanguinis* in the database to differentiate accurately these two closely related species
	Only one spectrum of *Propionibacterium acnes* or *Bacillus cereus* present in the database is not enough to be representative of the true diversity of *P. acnes* and *B. cereus* profiles
Taxonomical discordances	*Stenotrophomonas maltophila* misidentified as *Pseudomonas hibiscicola*, which is an invalid name for *S. maltophila*
	Agrobacterium tumefaciens is synonymous of *Rhizobium rhizogenes*
Insufficient protein signal	Yeasts require a protein extraction procedure to be correctly identified
Difficult to lyse cell wall structures	Pneumococci as well as most strains of *Haemophilus influenzae* and
Small amount of material sample	*Klebsiella pneumoniae* possess a capsule which prevents efficient lysis and results to poor spectral quality
	Actinomyces, *Gemella*, *Nocardia*, and *Streptomyces* species usually display weak protein signals.
	Better signal for *Enterobacteriaceae* grown on blood agar vs. MacConkey agar

"Rapid and simple *Shigella* and *E. coli* differentiation by MALDI-TOF using the VITEK® MS platform"

M. Arsac[1], V. Monnin[2], P. Bourne-Branchu[2], D. Pincus[3], H. Dwivedi[3], G. Devulder[3], G. Durand[2], A. van Belkum[2], V. Girard[2]

[1]*biomerieux, Marcy, France*

[2]*biomerieux, La Balme les Grottes, France*

[3]*biomerieux, Hazelwood, USA*

Objectives : *Shigella* species and *E.coli* are very closely related and their differentiation is needed from a clinical and veterinary perspective. *Shigella* species are always considered pathogenic whereas *E.coli* can be either pathogenic or part of the commensal flora. *Shigella spp* and *E.coli* are to date difficult to distinguish using MALDI-TOF MS. Tedious and time-consuming biochemical and serological methods are conventionally used and their differentiation remains a diagnostic challenge. The objective of this study was to set up a simple MALDI TOF MS method that could be implemented routinely in the laboratory allowing to distinguish these closely related species.

Methods :In this study, 106 well characterized strains of *Shigella* and *E. coli* including the pathogenic serovar 0157 were used to acquire 400 MALDI TOF MS spectra using a simple extraction procedure. After processing of the spectra, a predictive identification model was built and discriminative peaks were identified. Data exploration was also performed using multi-dimensional scaling (MDS).

Results : An estimation of performance by cross-validation and data exploration via MDS showed that 100% of *E. coli* 0157 strains were well identified at the serogroup level. Non-0157 *E. coli* and the four *Shigella* species (S. *boydi, S. dysenteriae, S. flexneri and S. sonnei)* were identified to the species level in 82% ,89%,90%,100% and 95% of the cases, respectively. Several discriminative peaks allowing the differentiation of the species were also highlighted. The validation of the prediction model on an external dataset of 62 Shiga-toxin producing *E. coli* (STEC) strains from different serogroups (excluding 0157) showed that 100% of the strains could be identified to the species level. However, identification at the serogroup level was not possible.

Conclusions : This study showed that the closely related *Shigella* spp and *E. coli* can be distinguish at the species level using MALDI TOF MS. In this study, it was not possible to distinguish serogroups, with 0157 being the single positive exception. This finding could be of great importance in the management of outbreaks and in epidemiological and surveillance studies

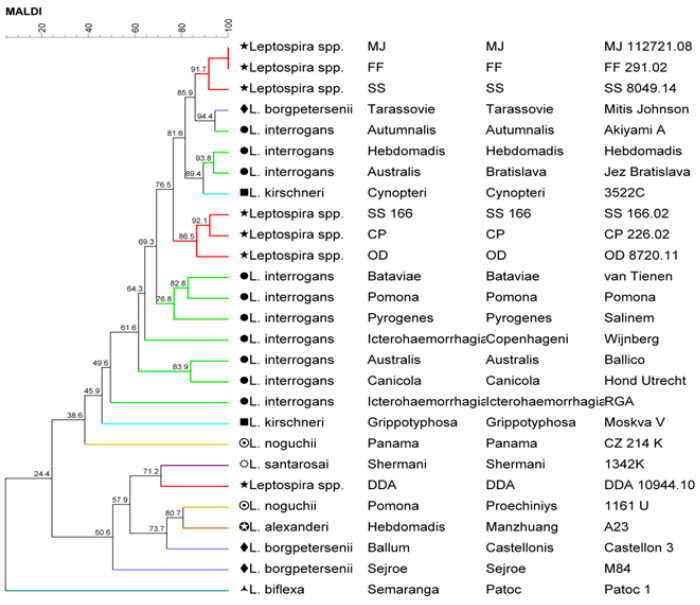
ECCMID 2015

Nadgradnje knjižnic

Bruker MALDI Biotyper

Možnost izdelave lastnih knjižnic

- Polna ekstrakcija izolata
- Ustrezna kalibracija
- Posnetek približno 24 spektrov
- Za izdelavo MSP se lahko uporabi samo kvalitetne spektre → nujna ustrezna obdelava spektrov s Flex Analysis
- Izdelava MSP z BioTyper software



D. Podgoršek, doktorska disertacija, 2017

Nadgradnje knjižnic

-> Prosto dostopne zbirke (open source libraries)

-> Komercialne prilagojene (custom) knjižnice

AnagnosTec SARAMIS™ ReferenceSpectra

>3000 vrst

-> Kontrola kvalitete (QC) in zanesljivost ?

-> FDA ?

Knjižnice & bakterije s posebnim statusom

Bruker Secure library

Bacillus anthracis (problem ločevanja od *B. cereus*)
Brucella melitensis
Burkholderia mallei
Burkholderia pseudomallei
Clostridium botulinum A, B, C, D, E, F, G
Francisella tularensis
Salmonella Paratyphi
Salmonella Typhi
Shigella dysenteriae
Vibrio cholerae
Yersinia pestis


RAPID COMMUNICATIONS

Fatal anthrax infection in a heroin user from southern Germany, June 2012

T Holzmann (thomas.holzmann@klinik.uni-regensburg.de)[1], D Frangoulidis[2], M Simon[1], P Noll[2], S Schmoldt[2], M Hanczaruk[2], G Grass[3], M Pregler[4], A Sing[4], S Hörmansdorfer[4], H Bernard[5], R Grunow[6], R Zimmermann[6], W Schneider-Brachert[1], A Gessner[1], U Reischl[1]

JCM
Journals.ASM.org

FAST-TRACK COMMUNICATION

Importance of Using Bruker's Security-Relevant Library for Biotyper Identification of *Burkholderia pseudomallei*, *Brucella* Species, and *Francisella tularensis*

Scott A. Cunningham,[a] Robin Patel[a,b]

-> S standardno knjiznico identifikacija **NI možna** ne glede na kvaliteto spektra

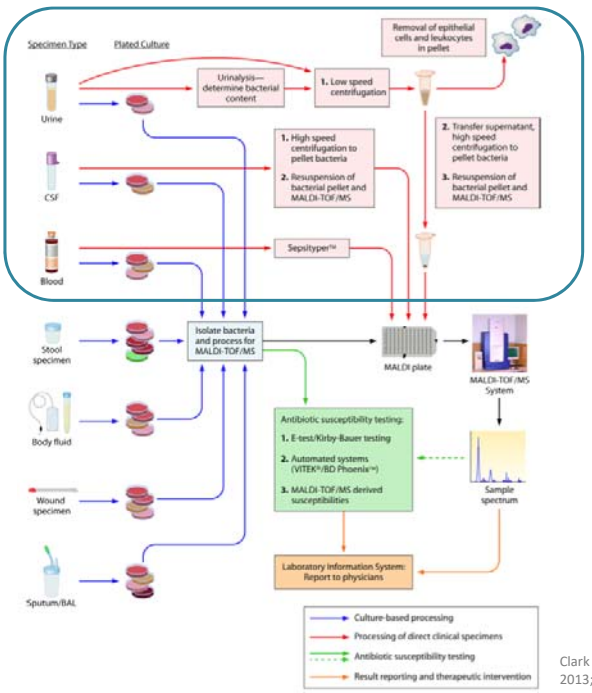
-> Majhno število testiranih izolatov

-> S standardno knjižnico ni napacnih identifikacij + NI OPOZORILA
pazljivo pri *Burkholderia thailandensis*

-> SR library Bruker Daltonics → BSL3 patogeni

Bakterijski izolat	Score/ID s standardno knjiznico	ID s SR knjiznico
Brucella melitensis (n=6)/Brucella suis (n=3)	<1,7 (ni zanesljiva ID)	5 izolatov B. melitensis (vmes 1 B. suis)
Francisella tularensis (n=9)	<1,7 (ni zanesljiva ID)	7 izolatov Francisella tularensis, 2 Francisella spp.
Burkholderia pseudomallei (n=2)	1,954 B. thailandensis	B. mallei/B.pseudomallei

MALDI-TOF & IDENTIFIKACIJA BAKTERIJ NEPOSREDNO IZ KUŽNIN



Clark AE et al. Clin Microbiol Rev. 2013;26:547–603.

Neposredna identifikacija iz pozitivnih hemokultur

Odstranitev humanih celic iz pozitivne hemokulture

Liziranje celic:

- Detergent: Na dodecyl sulfate, **saponin**, Tween 80
- Soli: amonijev klorid

Ločevanje s centrifugiranjem:

- Serumska epruveta z gelom
- Diferencialno centrifugiranje
- Komercialni kit **MALDI Sepsityper®** (Bruker Daltonik, Nemčija)

Koncentrirani mikroorganizmi iz pozitivne hemokulturne stekleničke

Sediment → neposredna ID

Različni ekstrakcijski postopki → ID

Marinach-Patrice in sod. PLoS One 2010, Ferroni in sod. JCM 2010, Martiny in sod. EJCMID 2012, Stevenson JCM 2010, Moussaoui in sod. CMI 2010, Spanu JCM 2010, Prod'hom in sod. JCM 2010, Christner JCM 2010, Schubert J Mol Diag JCM 2010, Juiz EJCMID 2012, La Scola PLoS One 2009, Ferreira CMI 2011, Schmidt EJCMID 2012, March–Rosselló EJCMID 2013

Review Article

Rapid Identification of Pathogens in Positive Blood Culture of Patients with Sepsis: Review and Meta-Analysis of the Performance of the Sepsityper Kit

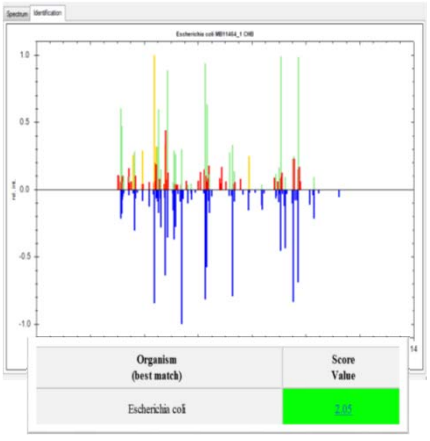
Nils G. Morgenthaler[1,2] and Markus Kostrzewa[2]

Int J Microbiol. 2015

-> 21 člankov
-> skupno 3320 pozitivnih hemokulturnih stekleničk
-> 80% zanesljiva ID na nivoju vrste
gramnegativne bakterije 90%
grampozitivne bakterije 76%
kvasovke 66%

Neposredna identifikacija iz pozitivnih hemokultur - Sepsityper modul

Standard



Sepsityper modul

Posebna analiza masnih spektrov in prilagojen algoritem ID -> preverja, ali je možno, da gre za mešano kulturo

27th ECCMID Vienna, Austria 22 – 25 April 2017

25 April 2017, 13:54 - 14:04
OS1028

Comparison of bacterial identification directly from positive blood culture bottles by MALDI-TOF using standard MALDI Biotyper Compass and Sepsityper module

Mateja Pirs*[1], Damjana Barbic[2], Manica Mueller-Premru[3]

795/856 (92.9%) bakteriemija z 1 vrsto
61/856 (7.1%) mešana bakteriemija (kultura)
24/856 (2.8%) mešana bakteriemija vidna v gramskem razmazu

61/856 (7.1%) mešanih bakteriemij:

- Pravilna ID obeh patogenov v 4 (6.6%) primerih
- ID ene vrste v 42 (68.9%) primerih
- ID neuspešna v 14 (22.9%) primerih
- Nepravilna ID 1 primer

24/856 (2.8%) mešanih bakteriemij vidnih v gramskem razmazu:

- Pravilna ID obeh patogenov v 2 (8.3%) primerih
- ID ene vrste v 15 (62.5%) primerih
- ID neuspešna v 7 (29.2%) primerih

27th ECCMID Vienna, Austria 22 – 25 April 2017

25 April 2017, 13:54 - 14:04
OS1028

Comparison of bacterial identification directly from positive blood culture bottles by MALDI-TOF using standard MALDI Biotyper Compass and Sepsityper module

Mateja Pirs*[1], Damjana Barbic[2], Manica Mueller-Premru[3]

ZAKLJUČKI

- **Lažna ID mešane bakteriemije** → lahko se po nepotrebno razširi spekter antibiotične terapije (EC/KPN), ali uvede zdravljenje (KNS/SA)
- **Zgrešene dejanske mešane bakteriemije**
- **Lahko prednost pred standardnim modulom**

Testirana verzija modula Compass Explorer v4.1.40 še ni primerna za rutinsko uporabo. Izkušeni uporabniki jo lahko uporabljajo pri mešani bakteriemiji v gramskem razmazu

Neposredna identifikacija bakterij iz urina

-> Preprečimo nepotrebno/neustrezno antibiotično terapijo
-> Presejanje: prisotnost bakterij v urinu, okužba sečil?
-> Različni protokoli za odstranitev levkocitov in ID z MALDI - TOF:
volumen vzorca, 1-15 ml , dodajanje SDS, predhodna kratka kultivacija, filtracija, centrifugiranje
-> Moteči dejavniki - defenzini

ZAKLJUČKI
-> -G > +G
-> meja detekcije 100.000 CFU/ml
-> 70 % monomikrobnih okužb ustrezna ID
-> *E. coli/Shigella* spp ?

Burillo A et al. Plos one 2014; 2 Rossello GAM et al APMIS 2013; 3 Sanchez-Juanes F et al. J Clin Microbiol 2014; 4 Veron L et al. Eur J Clin Microbiol ID 2015; 5 Kim Y et al. Ann Lab Med 2015; 6 Wang XH et al. J Micro Method 2013

Neposredna identifikacija bakterij iz likvorja

-> Primerno za likvorje s pozitivnim gramskim razmazom

-> Nezadostni volumen kužnine in prenizko bakterijsko breme v likvorju za neposredno identifikacijo z MALDI -TOF

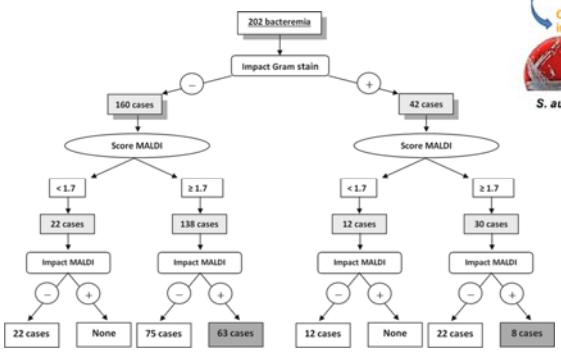
Bishop B et al, The use of Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for rapid bacterial identification in patients with smear-positive bacterial meningitis, Clin Microbiol Infect 2017

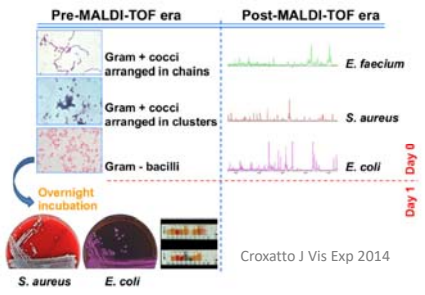
POSLEDICE IDENTIFIKACIJE BAKTERIJ NEPOSREDNO IZ KUŽNIN Z MALDI – TOF ZA POTEK ZDRAVLJENJA

Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the Clinical Management of Patients With Gram-negative Bacteremia: A Prospectiv Observational Study

Olivier Clerc,[1] Guy Prod'hom,[2] Christelle Vogne,[2] Alain Bizzini,[2] Thierry Calandra,[1] and Gilbert Gre

Clinical Infectious Diseases 2013;56(8):1101–7





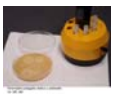
Croxatto J Vis Exp 2014

Gramski razmaz 20,8%
ID z MALDI TOF 35,1% -> modifikacija antibiotične terapije pri bakteriemiji

MALDI-TOF & DOLOČANJE OBČUTLJIVOSTI ZA ANTIBIOTIKE

Določanje občutljivosti za antibiotike

EUCAST: Občutljivost določamo glede na rezultat fenotipskega testiranja, meritev interpretiramo glede na opredeljene mejne vrednosti



Fenotipsko testiranje - klinični kriteriji:

- **Izolat je občutljiv**
- **Izolat je odporen**

Opredeljevanje prisotnosti rezistenčnega mehanizma:

- Rezistenčni mehanizem dokazan → pričakujemo, da je izolat klinično odporen
- Rezistenčnega mehanizma ne dokažemo → ne moremo predvideti, če je izolat klinično odporen ali ne

MALDI - TOF & AST

Hitro določanje ekvivalenta MIK za opredelitev S/I/R

Detekcija rezistenčnega mehanizma

-> Dokazovanje encimske aktivnosti na podlagi spremembe teže molekule (pr. hidroliza meropenema)

-> Detekcija celičnih komponent, ki sodelujejo pri mehanizmu odpornosti

Detekcija epidemioloških markerjev -> detekcija klonov z določeno obliko odpornosti

MALDI - TOF & AST

Hitro določanje ekvivalenta MIK za opredelitev S/I/R

-> Klasična fenotipska metoda: opredelitev MIK

-> **MALDI-TOF**

Minimalna koncentracija antibiotika, pri kateri pride do spremembe profila bakterije

Minimalna efektivna koncentracija

Quantitative Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Resistance Detection

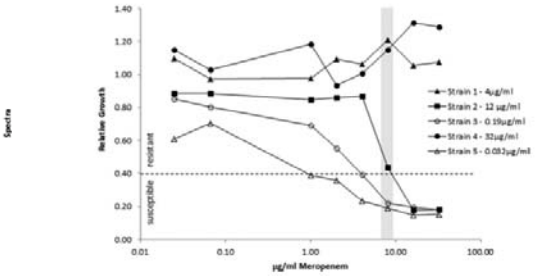
Christoph Lange,[a] Sören Schubert,[b] Jette Jung,[b] Markus Kostrzewa,[a] Katrin Sparbier[a]

Journal of Clinical Microbiology p. 4155–4162 December 2014

Inkubacija bakterij v gojišču z in brez antibiotika -> primerjava spektrov
Uporaba optimalnega gojišča za rast bakterij

Kratka inkubacija 2,5 – 3 ure → liza celic + dodatek internega standarda → MALDI-TOF → primerjava spektrov bakterij, ki so rasle z in brez antibiotika → ocena odpornosti glede na primerjavo hitrosti razmnoževanja bakterij

MBT ASTRA



MALDI - TOF & AST

Hitro določanje ekvivalenta MIK za opredelitev S/I/R

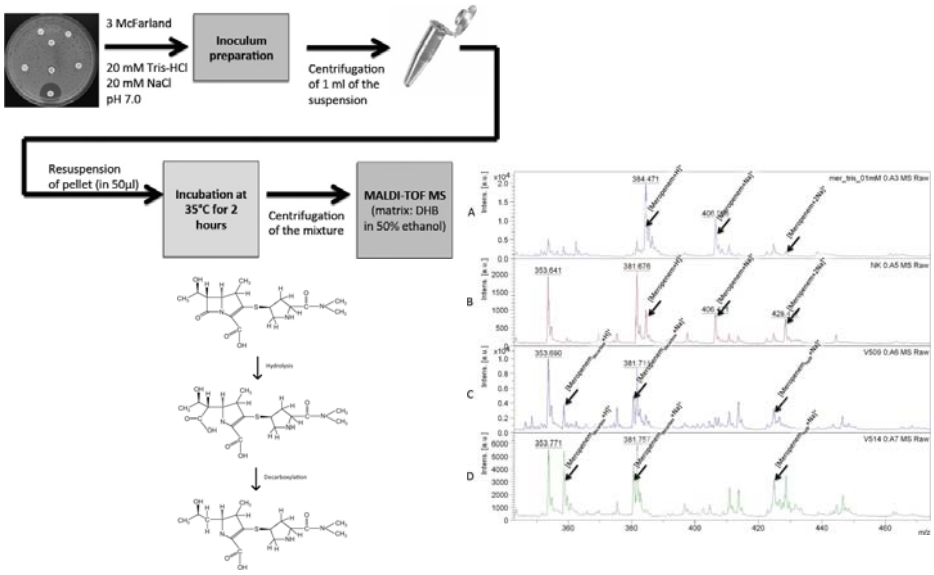
Detekcija rezistenčnega mehanizma

-> Dokazovanje encimske aktivnosti na podlagi spremembe teže molekule (pr. hidroliza meropenema)

-> Detekcija celičnih komponent, ki sodelujejo pri mehanizmu odpornosti

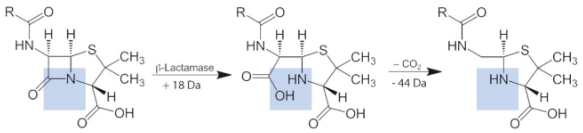
Detekcija epidemioloških markerjev -> detekcija klonov z določeno obliko odpornosti

Detekcija encimske aktivnosti



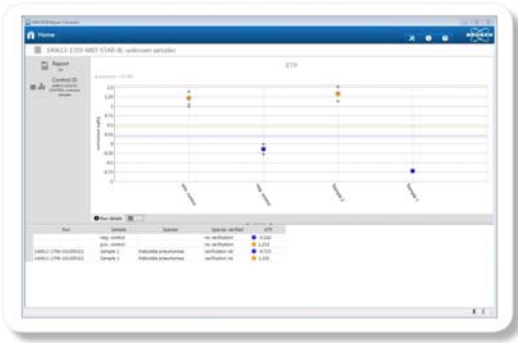
Hrabak in sod. JCM 2012

STAR-BL modul (Bruker)



Hydrolysis of an antibiotic's β-lactam ring leads to mass shifts that can easily be detected by MALDI-TOF mass spectrometry

STAR-BL-Carba kit



Color-coded plot indicating presence (orange) or absence (blue) of β-lactamase activity in bacterial strains

Detekcija celične komponente rezistenčnega markerja

Anal Chem. 2002 Nov 1;74(21):5487-91.

Identification of Staphylococcus aureus and determination of its methicillin resistance by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Du Z[1], Yang R, Guo Z, Song Y, Wang J.

Author information

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Keywords:
MRSA
Staphylococcus aureus
MALDI-TOF MS
PSM-mec
Class A mec gene complex
SCCmec

the genomes of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) strains. This peptide is excreted by *agr*-positive strains, which represent about 89% of the strains of our collection and can be identified by the presence of delta toxin in mass spectrometry. The presence of the peptide in the MALDI-TOF MS spectra of whole cells was proved by a knock-down experiment employing a clone that expressed antisense RNA to *psm-mec*. Furthermore, evaluation of a collection of clinical *agr*-positive MRSA and MSSA isolates and type strains showed that, using a detection window of m/z 2411–2419, the PSM-mec is detected by mass spectrometry of whole cells with a sensitivity of 0.95 and a specificity of 1, thereby enabling rapid identification of a subgroup of MRSA with a method that is used during routine identification procedures.

Blood culture bacterial pellet

Gram staining ➡ **≤1h**
> 99% accurate

MALDI-TOF MS ➡ **≤1h**
> 99% accurate

Antibiotic suceptibility testing ➡ **16-24h**
> 99% accurate

MALDI:

- **Detekcija PSM-mec+ sevov MRSA (hkrati z ID)**
- **STAR-BL Carba (dodatna izvedba testa + rekalibracija)**
- **nekomercialne metode za detekcijo ESBL (analogno STAR-BL) (dodatna izvedba testa + rekalibracija)**

Croxatto J Vis Exp 2014

MALDI-TOF &
TIPIZACIJA BAKTERIJ

Microbial Typing by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: Do We Need Guidance for Data Interpretation?

Sébastien Spinali,[a] Alex van Belkum,[a] Richard V. Goering,[b] Victoria Girard,[a] Martin Welker,[a] Marc Van Nuenen,[c] David H. Pincus,[d] Maud Arsac,[e] Géraldine Durand[a]

Journal of Clinical Microbiology March 2015

Serotipizacija -> posamezni antigeni

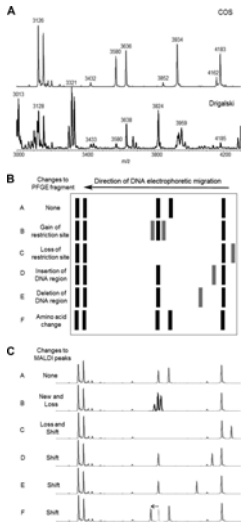
Genotipizacija -> DNA

MALDI-TOF tipizacija -> rutinski MS - znotrajcelični proteini

Microbial Typing by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: Do We Need Guidance for Data Interpretation?

Sébastien Spinali,[a] Alex van Belkum,[a] Richard V. Goering,[b] Victoria Girard,[a] Martin Welker,[a] Marc Van Nuenen,[c] David H. Pincus,[d] Maud Arsac,[e] Géraldine Durand[a]

Journal of Clinical Microbiology March 2015



A. MALDI-TOF MS spekter iz enega izolata E.coli na 2 različnih gojiščih (Columbia agar, Drigalski agar)

B. Teoretični PFGE gel (ocena 20-30 DNA fragmentov)
Tenoverjevi kriteriji: do 3 različni fragmenti → epidemiološka povezanost

C. MALDI-TOF MS analogi za PFGE gel (analiza 100 masnih vrhov -> 15 masnih vrhov razlike = epidemiološka povezanost?)

IDENTIFIKACIJA

-> Uporaba različnih gojišč (krvni agar, čokoladni agar…)

-> Neodvisno od izpostavljenosti antibiotikom (selektivna gojišča)

-> Različno trajanje kultivacije

-> Tipično zadošča 5-10 značilnih masnih vrhov

-> Statistična obdelava podatkov razvita

(SUB)TIPIZACIJA

-> **Problem standardizacije pri pridobivanju masnega spektra, številni vplivi!**

-> Enako gojišče za vse testirane izolate, čas in pogoji rasti

-> Enaka količina bakterij na tarči

-> Enak matriks

-> Enak protokol ekstrakcije proteinov

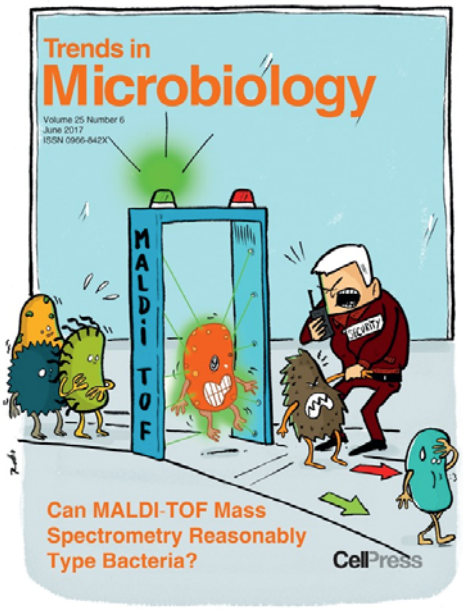
-> Potrebnih več značilnih vrhov (≈> 20)

-> Problem statistične obdelave rezultatov (Biotyper, Bionumerics, paket R)

Review

Can MALDI-TOF Mass Spectrometry Reasonably Type Bacteria?

Marlène Sauget,[1,2,3,*] Benoît Valot,[3] Xavier Bertrand,[1,2,3] and Didier Hocquet[1,2,3]



Trends in Microbiology, June 2017, Vol. 25, No. 6

Zaključki

1. **Identifikacija bakterij z MALDI – TOF -> revolucija**
2. **Dopolnjevanje in izboljšave knjižnic, odprava določenih napak**
3. **Pomen identifikacije bakterij neposredno iz kužnin za zdravljenje bolnikov**
4. **Številne aplikacije in študije za ugotavljanje odpornosti proti antibiotikom, na voljo nekateri komercialni kiti, vendar še nepreverjeni**
5. **Izkušnje uporabnikov s tipizacijo bakterij z MALDI – TOF so različne, nujna standardizacija**