Ongoing revolution in clinical microbiology: current status and future perspectives

Mario Poljak

Institute of Microbiology and Immunology
Faculty of Medicine, University of Ljubljana, Slovenia

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,...)

Molecular diagnostic systems 1.0

Molecular diagnostic systems 2.0+

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

NeuMoDx (Qiagen)

Cobas 6800/8800 (Roche)

Panther Fusion (Hologic)

DxN Veris (Beckman Coulter)

Alinity m (Abbott)

revolution ?

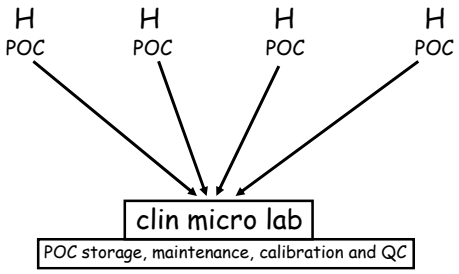
point-of-care tests and 24/7 concept

faster

cheaper

24/7

Two testing places – evolving concept



POC = point-of-care

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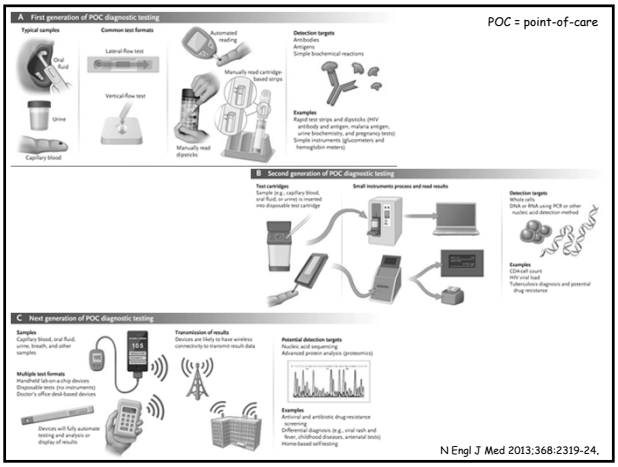


N Engl J Med 2013;368:2319-24.

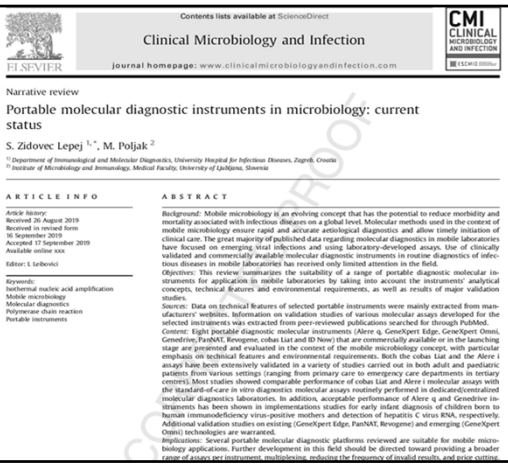
desire is to have self-contained, fully integrated sample-to-report devices that accept raw, untreated specimens, perform all of the molecular steps, and provide interpreted test results in < 1 h

Selected compact "sample in-results out" molecular diagnostic devices

Cepheid GeneXpert
Roche cobas Liat System
Alere iSystem
Luminex ARIES
Atlas Genetics
Enigma Diagnostics
Micronics
Cirrus Dx T-COR 8
BioFire FilmArray
Nanosphere Verigene SP
QuantuMDx
Janssen Diagnostics
Rheonix Encompass Optimum

GenMark Dx eSensor
Veredus VereChip
Great Basin Portrait
Focus Dx Simplexa/3M Cycler
QuidelSavanna & Solana
Meridian Illumigene
BD Max System
ELITe InGenius Systems
Biomeme
Fluoresentric, Inc.
GeneWEAVE VivoDx

U.S. market in 2019 for POC - $18 billion dollar !



Contents lists available at ScienceDirect
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Narrative review

Portable molecular diagnostic instruments in microbiology: current status

S. Zidovec Lepej [1,*], M. Poljak [2]
[1] Department of Immunological and Molecular Diagnostics, University Hospital for Infectious Diseases, Zagreb, Croatia
[2] Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Slovenia

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ABSTRACT
Background: Mobile microbiology is an evolving concept that has the potential to reduce morbidity and mortality associated with infectious diseases on a global level. Molecular methods used in the context of mobile microbiology ensure rapid and accurate aetiological diagnostics and allow timely initiation of clinical care. The great majority of published data regarding molecular diagnostics in mobile laboratories have focused on emerging viral infections and using laboratory-developed assays. Use of clinically validated and commercially available molecular diagnostic instruments in routine diagnostics of infectious diseases in mobile laboratories has received only limited attention in the field.
Objectives: This review summarizes the suitability of a range of portable diagnostic molecular instruments for application in mobile laboratories by taking into account the instruments' analytical concepts, technical features and environmental requirements, as well as results of major validation studies.
Sources: Data on technical features of selected portable instruments were mainly extracted from manufacturers' websites. Information on validation studies of various molecular assays developed for the selected instruments was extracted from peer-reviewed publications searched for through PubMed.
Content: Eight portable diagnostic molecular instruments (Alere q, GeneXpert Edge, GeneXpert Omni, Genedrive, FamNAT, Revogene, cobas Liat and ID Now) that are commercially available or in the launching stage are presented and evaluated in the context of the mobile microbiology concept, with particular emphasis on technical features and environmental requirements. Both the cobas Liat and the Alere i assays have been extensively validated in a variety of studies carried out in both adult and paediatric patients from various settings (ranging from primary care to emergency care departments in tertiary centres). Most studies showed comparable performance of cobas Liat and Alere i molecular assays with the standard-of-care in vitro diagnostics molecular assays routinely performed in dedicated/centralized molecular diagnostics laboratories. In addition, acceptable performance of Alere q and Genedrive instruments has been shown in implementations studies for early infant diagnosis of children born to human immunodeficiency virus-positive mothers and detection of hepatitis C virus RNA, respectively. Additional validation studies on existing (GeneXpert Edge, FamNAT, Revogene) and emerging (GeneXpert Omni) technologies are warranted.
Implications: Several portable molecular diagnostic platforms reviewed are suitable for mobile microbiology applications. Further development in this field should be directed toward providing a broader range of assays per instrument, multiplexing, reducing the frequency of invalid results, and price cutting.

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

J Clin Microbiol 2016;54:815

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]

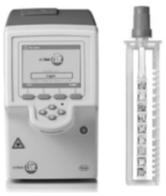
cobas Liat strep A assay vs. *S. pyogenes* LightCycler PCR assay

sensitivity = 100%

specificity = 98.3 %

positive predictive value = 97.7%

negative predictive value = 100.0%



Evaluation of a routine point-of-care intervention for early infant diagnosis of HIV: an observational study in eight African countries

Lancet HIV 2019;6:e373-81

Flavia Bianchi, Jennifer Cohn*, Emma Sacks, Rebecca Bailey, Jean-Francois Lemaire, Rhoderick Machekano, on behalf of the EGPAF POC EID Study Team†*

POC testing in 339 health-care facilities:

- 2,875 infants exposed to HIV tested with conventional testing methods
- 18,220 infants tested with POC testing

the return of results to caregivers within 30 days: 18.7% vs. 98.3%

the median time from sample collection to return of results: 55 days vs. 0 days

the median time from sample collection to ART initiation: 49 days vs. 0 days

infants with HIV initiating antiretroviral therapy within 60 days: 43.3% vs. 92.3%

the cost per test result returned within 30 days: $131 vs. $27

Where is my instrument ?

universal instruments providing electricity and software

Lab-on-a-USB key

microfluidic devices integrated with USB key data storage devices

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

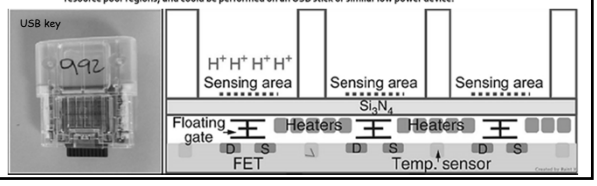
analysis transmitted to central databases for shared use and meta-processing

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.



Journal Pre-proof

Narrative Review

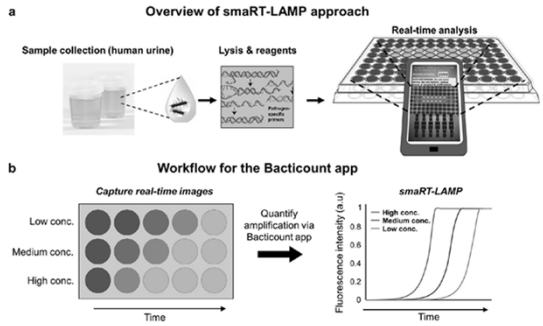
Smartphones as mobile microbiological laboratories

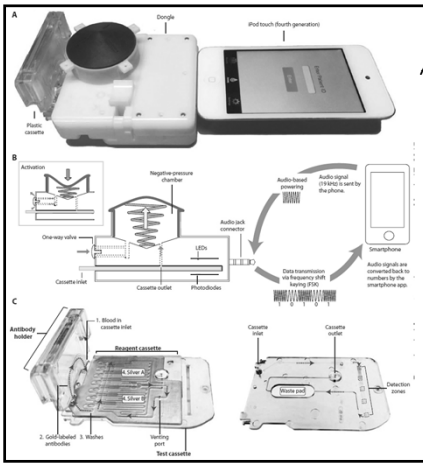
David S. Y. Ong,[1,2] Mario Poljak[3]

Smartphone-based pathogen diagnosis in urinary sepsis patients

EBioMedicine 2018;36:73-82

Lucien Barnes [a,1], Douglas M. Heithoff [a,1], Scott P. Mahan [a], Gary N. Fox [b], Andrea Zambrano [c], Jane Choe [d], Lynn N. Fitzgibbons [c], Jamey D. Marth [a,e], Jeffrey C. Fried [f], H. Tom Soh [g,*], Michael J. Mahan [a,**]





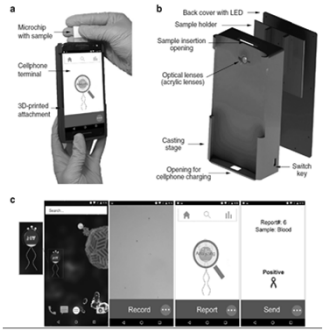
A smartphone dongle as point-of-care device

Sci Trans Med 2015; 7: 273re1

DNA engineered micromotors powered by metal nanoparticles for motion based cellphone diagnostics

Mohamed Shehata Draz[1,2,3], Kamyar Mehrabi Kochehbyoki@[1], Anish Vasan[1], Dheerendranath Battalapalli[1], Aparna Sreeram[1], Manoj Kumar Kanakasabapathy[1], Shantanu Kallakuri@[1], Athe Tsibris[2,4], Daniel R. Kuritzkes[2,4] & Hadi Shafiee@[1,2]

Nat Commun 2018;9:4282



platform that integrates cellphone-based optical sensing, loop-mediated isothermal DNA amplification and micromotor motion

qualitative detection of HIV-1 (n=54) with 99.1% specificity and 94.6% sensitivity at a clinically relevant threshold value of 1,000 copies/ml.

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delivery of samples or standard POC instruments and reagents by drones

Narrative Review

Use of drones in clinical microbiology and infectious diseases: current status, challenges and barriers

M. Poljak*, A. Šterbenc

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

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Reuters Health Information

REUTERS HEALTH INFORMATION

Rwanda to Start Using Drones to Supply Vaccines, Blood in August

By Clement Uwiringiyimana

May 16, 2016

Rwanda is the first country that has sought to integrate drones into its health service with drones used to deliver blood; launched in 2016

4,100 units of blood delivered in the first year; delivery time 15-45 minutes; 40% of the blood for postpartum hemorrhaging; 40% for treatment of severe malaria

Buzz as World's Biggest Drone Drug Deliveries Take Off in Tanzania

By Kizito Makoye

August 30, 2017

Tanzania launched the world's largest drone delivery network in January 2018

more than 1,000 health facilities across the Tanzania connected

delivery of blood, vaccines and malaria and HIV/AIDS drugs

modified Zipline International drones flying at 100 km (62 miles) per hour; drones parachuting blood and medicines with biodegradable parachutes

Can Unmanned Aerial Systems (Drones) Be Used for the Routine Transport of Chemistry, Hematology, and Coagulation Laboratory Specimens?

PLoS One 2015;10:e0134020

Timothy K. Amukele[1,2nd]*, Lori J. Sokoll[1na], Daniel Pepper[3rd], Dana P. Howard[4nc], Jeff Street[4nd]

three paired samples obtained from 56 adult volunteers; chemistry, hematology, and coagulation testing

168 samples held stationary vs. 168 samples flown in the UAS (6-38 minutes)

33 of the most common chemistry, hematology, and coagulation tests performed

a mean difference of 3.2% for glucose and <1% for other analytes

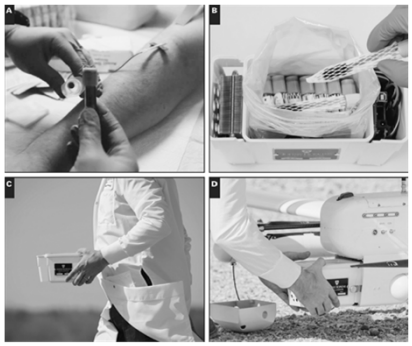
only bicarbonate did not meet the strictest performance criteria.

transportation of laboratory specimens via small UASs does not affect accuracy of routine chemistry, hematology, and coagulation tests results

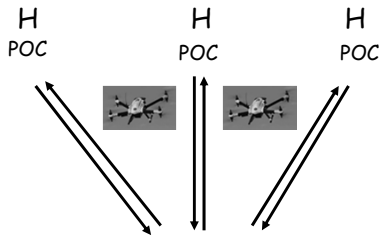
Drone Transport of Chemistry and Hematology Samples Over Long Distances

Am J Clin Pathol 2017;148:427-35

Timothy K. Amukele, MD, PhD,[1] James Hernandez, MD,[2] Christine LH. Snozek, PhD,[2] Ryan G. Wyatt, FPC,[3] Matthew Douglas, MD,[1] Richard Amini, MD,[3] and Jeff Street[1]



Sample transport and rent-a-POC concept



POC storage, maintenance, calibration and QC

clin micro lab

POC = point-of-care

Optimizing a Drone Network to Deliver Automated External Defibrillators

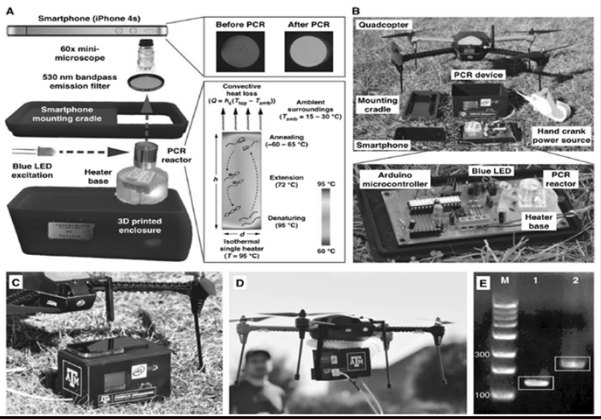
Circulation 2017;135:2454-65

Boutilier J. J., Brooks S. C., Byers A. J. A., Buick E. J., Zhan C., Schoellig A. P., Cheskes S., Morrison L. J., Chan T. C. Y.

Lab-on-a-drone

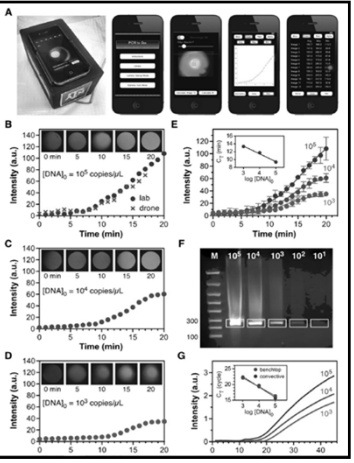
Anal Chem 2016; 88: 4651–4660

successful in-flight PCR amplification using convective thermocycling of two different DNA targets (16 min in-flight reaction time)



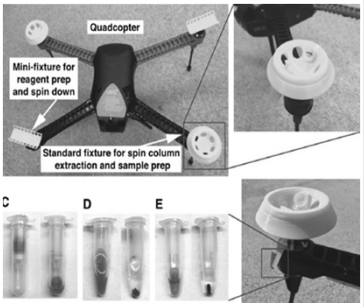
Lab-on-a-drone

time-resolved fluorescence detection and quantification using a smartphone camera and integrated image analysis app



Anal Chem 2016; 88: 4651–4660

Lab-on-a-drone



Drone-based centrifugation

benchtop centrifugation processes are challenging to miniaturize

by removing the quadcopter propellers and replacing them with 3D printed centrifuge rotors designed to fit the motor shaft threading

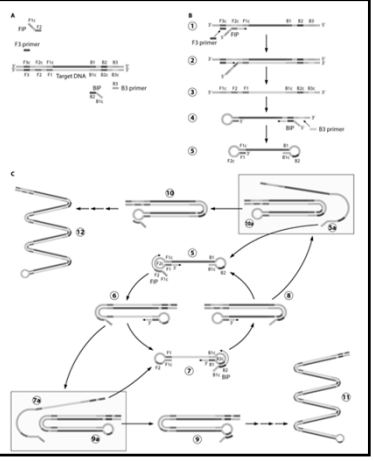
Drone-based sample preparation

standard centrifuge-based workflows allowed in lab-on-a-drone

3D printed attachments transform the quadcopter into a centrifuge capable of rotation speeds up to 10,000 rpm

standard column-based extraction of Dengue viral RNA from human serum yielded results on par with those obtained from samples processed using a benchtop centrifuge

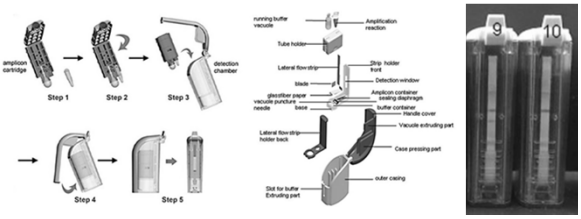
Loop-mediated isothermal amplification (LAMP)



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
- reagents stable at ambient temperature



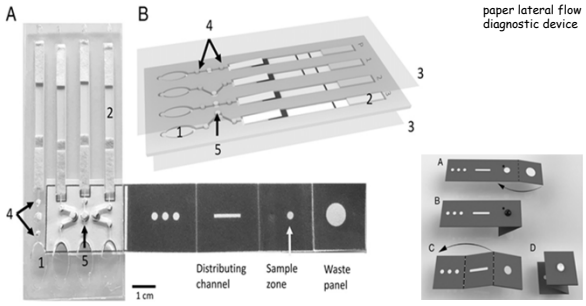
The Journal of Infectious Diseases 2010;201(S1):S65–S71

Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities

Julien Reboud[a,1], Gaolian Xu[b,1], Alice Garrett[a], Moses Adriko[c], Zhugen Yang[a], Edridah M. Tukahebwa[c], Candia Rowell[c], and Jonathan M. Cooper[a,2]

PNAS 2019; 116:4834-4842

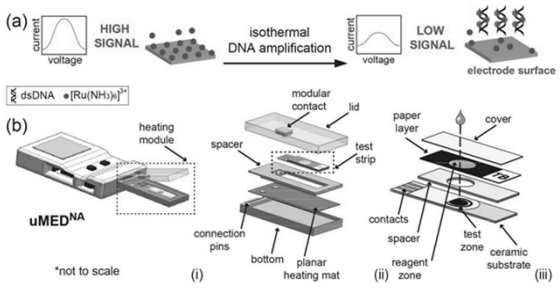
paper lateral flow diagnostic device



Handheld isothermal amplification and electrochemical detection of DNA in resource-limited settings

Maria-Nefeli Tsaloglou[a,c], Alex Nemiroski[a], Gulden Camci-Unal[a], Dionysios C. Christodouleas[a], Lara P. Murray[a], John T. Connelly[c], George M. Whitesides[a,b,c]

Anal Biochem 2018;543:116-21



the first fully integrated, POC device that combines isothermal DNA amplification with electrochemical detection on paper

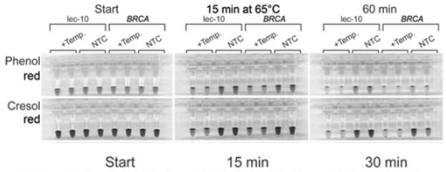
Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes

Nathan A. Tanner, Yinhua Zhang, and Thomas C. Evans Jr.
DNA Enzymes Division, New England Biolabs, Ipswich, MA

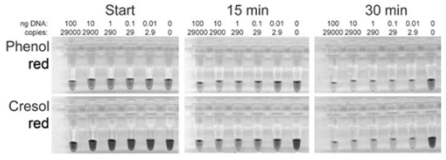
BioTechniques 58:59-68 (February 2015)

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

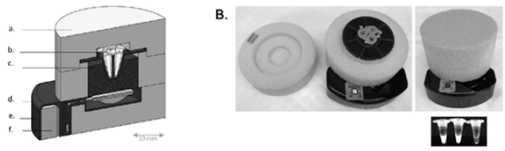


Single-use, electricity-free amplification device for detection of HIV-1

Kelly A. Curtis[a,*], Donna L. Rudolph[a], Daphne Morrison[a], Dylan Guelig[b], Steven Diesburg[b], David McAdams[b], Robert A. Burton[b], Paul LaBarre[b], Michele Owen[a]

[a] Laboratory Branch, Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Atlanta, GA 30329, USA
[b] PATH, 2201 Westlake Avenue, Suite 200, Seattle, WA 98121, USA

J Virol Methods 2016;237:132-7



non-instrumented nucleic acid amplification, single-used disposable (NINA-SUD) devices for the detection of HIV-1 in whole blood using reverse-transcription, loop-mediated isothermal amplification (RT-LAMP) with lyophilized reagents

NINA-SUD heating device harnesses the heat from an exothermic chemical reaction initiated by the addition of saline to magnesium iron powder

lyophilized HIV-1 RT-LAMP reagents stable at 30°C for up to one month

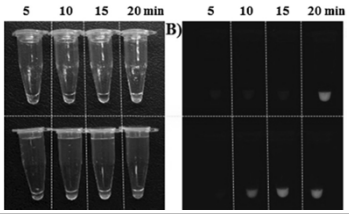
Ten-minute direct detection of Zika virus in serum samples by RT-LAMP

Paulo Felipe Neves Estrela[a], Geovana de Melo Mendes[a], Kézia Gomes de Oliveira[a], Alexandre Melo Bailão[b], Célia Maria de Almeida Soares[b], Nilson Antônio Assunção[c], Gabriela Rodrigues Mendes Duarte[a,*]

[a] Instituto de Química, Universidade Federal de Goiás, Goiânia, GO, 74690-900, Brazil
[b] Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, 74690-900, Brazil
[c] Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, São Paulo, SP, 04021-001, Brazil

RT-LAMP and visual detection of amplified products

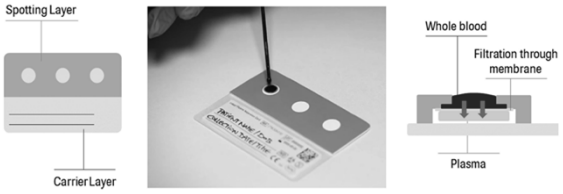
reaction thermally controlled with a thermoblock for 10 min at 72 °C



Separation of Plasma from Whole Blood by Use of the cobas Plasma Separation Card: a Compelling Alternative to Dried Blood Spots for Quantification of HIV-1 Viral Load

J Clin Microbiol 2019;57:e01336-18

Sergio Carmona,[a,b] Britta Seiverth,[c] Dieketseng Magubane,[b] Lucia Hans,[a,b] Matthias Hoppler[c]



revolution ?

MALDI-TOF mass spectrometry

(matrix assisted laser desorption ionization-time of flight mass spectrometry)

MALDI-TOF in clinical microbiology in 2019

speed (<3 min/isolate; 96 isolates/17 min); easy to perform; low cost

identification of bacterial/fungal isolates – revolution
(libraries regularly updated/enlarged)

direct identification of pathogens from clinical samples
(positive blood culture bottles, urine, positive clinical impact proved)

antimicrobial susceptibility testing and detection of resistance mechanisms
(limited number of resistance mechanisms can be identified or delayed response time, lack of established and standardized routines in detecting antimicrobial resistance, limited range of commercially available kits, lack of studies on the cost-effectiveness of incorporating resistancedetection by MALDI-TOF MS)

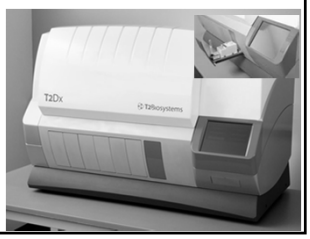
typing
(experiences vary substantially, standardization needed)

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
- result in 3-5 hours



Currently available assays:

T2 Candida Panel

T2 Bacteria Panel (ESKAPE pathogens)

T2 Resistance Panel (Ex-US launch end 2019)

T2 *Candida auris*

T2 Lyme

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

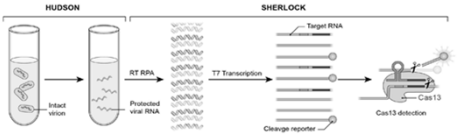
Rapid, inexpensive, and sensitive nucleic acid detection may aid point-of-care pathogen detection, genotyping, and disease monitoring. The RNA-guided, RNA-targeting clustered regularly interspaced short palindromic repeats (CRISPR) effector Cas13a (previously known as C2c2) exhibits a "collateral effect" 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.

CRISPR-Cas Biology and Its Application to Infectious Diseases

Jeffrey R. Strich,[a] Daniel S. Chertow[a,b]

[a]Critical Care Medicine Department, National Institutes of Health Clinical Center, Bethesda, Maryland, USA
[b]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

J Clin Microbiol 2019; 57:e01307-18.



Gootenberg et al. Science 2017;356:438-42
Gootenberg et al. Science 2018;360:439-44
Myhrvold et al. Science 2018;360:444-8
Roy et al. Front Genet 2018;9:240
Chen et al. Science 2018;360:436-9

SHERLOCK
DETECTR
CAMERA

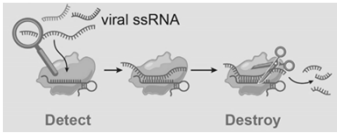
Programmable Inhibition and Detection of RNA Viruses Using Cas13

Molecular Cell 2019; in press.

Catherine A. Freije,[1,2,12,*] Cameron Myhrvold,[1,3,12,13,*] Chloe K. Boehm,[1] Aaron E. Lin,[1,2] Nicole L. Welch,[1,2] Amber Carter,[1] Hayden C. Metsky,[1,4] Cynthia Y. Luo,[1,3] Omar O. Abudayyeh,[1,5,6,7,8] Jonathan S. Gootenberg,[1,5,6,7,8] Nathan L. Yozwiak,[1,3] Feng Zhang,[1,5,6,7,10] and Pardis C. Sabeti[1,2,3,10,11,*]

CARVER - Cas13-assisted restriction of viral expression and readout

- an end-to-end platform that uses Cas13 to detect and destroy viral RNA
- programmably cleaves RNAs complementary to its CRISPR RNA (crRNA)
- Cas13's potent activity demonstrated against three distinct ssRNA viruses:
  - lymphocytic choriomeningitis virus
  - influenza A virus
  - vesicular stomatitis virus



revolution ?

<u>total</u> laboratory automation in bacteriology

Demonstrated advantages of automated specimen processing

<u>specimen processors compared to manual streaking:</u>

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

J Clin Microbiol 2009;47:1101-6.
J Clin Microbiol 2012;50:2732-6.
J Clin Microbiol 2014;52:796-802.
J Clin Microbiol 2015;53:2298-307.
J Med Microbiol 2018;67:1581-1588.

- decreased requirement for sub-culturing
- significant decrease in time to result, laboratory workload and costs

J Clin Microbiol 2015;53:2298-307.
J Clin Lab Anal 2018;32(5):e22373.

Current and future developments

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

- intelligent digital imaging

development of intelligent algorithms and expert systems with different applications

- microbial growth detection and quantification (BD Kiestra urine culture app)
- presumptive identification of species growing on chromogenic agar
  (WASPLab image analysis software for MRSA, VRE detection)

plates with negative results could reliably be automatically read and reported by the system to reduce the time and cost for large-volume screen laboratories

- fully automated disk diffusion AST

revolution ?

syndrome-specific testing

Syndrome-specific testing

comprehensive panels of probable pathogens causing a particular syndrome

highly multiplexed PCR platforms

one sample - multiple results

designed to directly probe specimens (respiratory, stool, CSF, blood, urogenital) and positive blood culture bottles for an array of microorganisms

can have significant impact on patient care and management

redefining the diagnosis of infectious disease ?

antimicrobial susceptibility testing
??????????????

genomic vs. phenotypic

revolution ?

genomic antimicrobial susceptibility testing
(targeted assays)

Genomic antimicrobial susceptibility testing

nucleic acid amplification 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

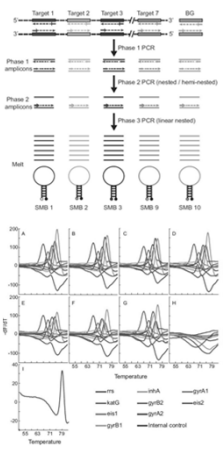
molecular testing will evolve versus syndrome-oriented multiplexed detection of pathogens including genomic AST

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

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 ?

genomic antimicrobial resistance testing

(whole genome sequencing)

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

N Eng J Med 2018;379:1403-15

The CRyPTIC Consortium and the 100,000 Genomes Project

- 10,209 isolates, genotypic predictions of the susceptibility of *M. tuberculosis* to first-line drugs
- resistance correctly predicted with 91.3% - 97.1% sensitivity
- susceptibility correctly predicted with 96.8% - 99.0% specificity
- England, the Netherlands, and New York discontinue phenotypic drug-susceptibility testing of isolates that are predicted by sequencing to be pan-susceptible to first-line drugs

Building the Framework for Standardized Clinical Laboratory Reporting of Next-generation Sequencing Data for Resistance-associated Mutations in *Mycobacterium tuberculosis* Complex

Clin Infect Dis 2019;69:1634–40

Jeffrey A. Tornheim,[1,a,©] Angela M. Starks,[2,a] Timothy C. Rodwell,[3,4] Jennifer L. Gardy,[3,4] Timothy M. Walker,[5] Daniela M. Cirillo,[6] Lakshmi Jayashankar,[3] Paolo Miotto,[6] Matteo Zignol,[10] and Marco Schito[11]

phenotypic antimicrobial susceptibility testing will remain core technology in clinical microbiology for multiple decades to come for majority of human pathogens

revolution ?

Emerging technologies for phenotypic antimicrobial susceptibility testing

Phenotypic antimicrobial susceptibility testing (short history)

agar plate methods

(large macroscopic colonies visible by naked eye required, time consuming)

↓

conventional turbidity methods

(10^7 CFU/mL required; bacterial must grow 16–20 h before reaching a detectable level)

↓

near future alternatives

(use of ultra sensitive alternative approaches for bacterial growth measurement)

Accelerate *Pheno* System
ID and AST direct from positive blood culture

FDA approved Accelerate Phen system and Accelerate PhenoTest BC kit for ID and AST testing of pathogens directly from positive blood culture samples in February 2017

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

New emerging technologies with great potential for phenotypic antibiotic susceptibility testing

flow cytometry
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

Forward laser light scatter technology (BacterioScan)

Rapid Antimicrobial Susceptibility Testing Using Forward Laser Light Scatter Technology

Randall T. Hayden,[a] Lani K. Clinton,[b] Carolyn Hewitt,[a] Terri Koyamatsu,[c] Yilun Sun,[d] Ginger Jamison,[a] Rosalie Perkins,[a] Li Tang,[d] Stanley Pounds,[d] Matthew J. Bankowski[d–g]
St. Jude Children's Research Hospital, Departments of Pathology[a] and Biostatistics,[d] Memphis, Tennessee, USA; Diagnostic Laboratory Services, Inc. (The Queen's Medical Center), Aiea, Hawaii, USA[b]; Department of Pathology and Department of Tropical Medicine, Medical Microbiology and Pharmacology[c] and Pathology Residency Program,[g] John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA
J Clin Microbiol 2016;54:2701-6

combines laser forward light scattering and optical density measurements of bacterial growth prior to visual assessment

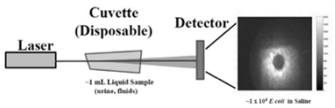
bacteriophage-mediated killing

limit of detection: 1x10^4 CFU/ml

undergoing FDA clinical trials

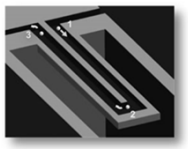
216Dx UTI System – UTI screening

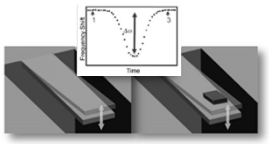
BacterioScan 3100R/AST/Dx



Resonate mass measurement (LifeScale - Affinity Biosensors)

- mechanically resonant structure with a microfluidic channel
- microbes suspended in broth and passed one by one through a microfluidic channel
- mass (and growth curve) measured by the change in resonate energy by ultra-high resolution weighing



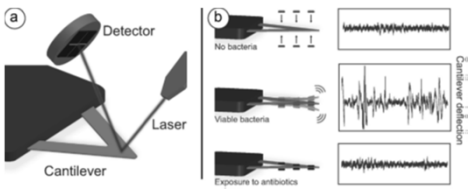


- FDA clinical trials started Q4 2016
- first FDA-approved test on market in 2020
- Gram negatives, 14 antibiotics

Microbial cell weighing by vibrating cantilevers + atomic force microscopy

Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections

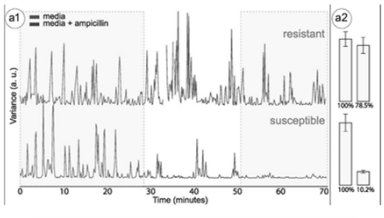
P. Stupar [1, 5], O. Opota [2, 5], G. Longo [1, 4], G. Prod'hom [2], G. Dietler [1], G. Greub [2, *, 5], S. Kasas [1, 3, 5]
Clinical Microbiology and Infection 23 (2017) 400–405

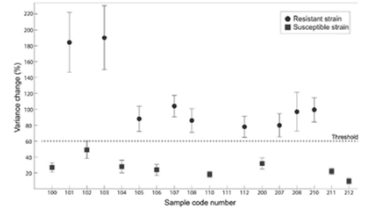


cantilevers containing small canals which facilitate microbial passage can be made to vibrate continuously

when metabolically active bacteria pass through, their weight (in the femtogram range) will cause a change in the frequency of cantilever movement

atomic force microscopy capable of detecting movements of biologic samples at the nanoscale





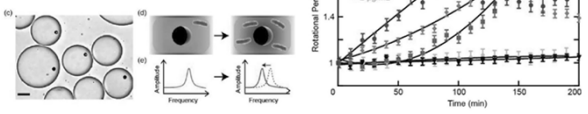
Clinical Microbiology and Infection 23 (2017) 400–405

Asynchronous magnetic bead rotation

Asynchronous magnetic bead rotation (AMBR) micro-viscometer for rapid, sensitive and label-free studies of bacterial growth and drug sensitivity

Anal Chem 2012;84:5250-6

Irene Sina[1,2,3], Theodore Albertson[1,4], Paivo Kinnunen[1,5], David N. Breslauer[3], Brandon H. McNaughton[1,2,3], Mark A. Burns[1], and Raoul Kopelman[1,2,4,5]



when magnetic beads are brought into a revolving magnetic field, they self-assemble and assume a specific rotational spin - the frequency of rotation can be influenced by the binding of bacteria

if the beads are equipped with a ligand that specifically captures bacterial cells, the rotation of the beads changes at the moment of capture - this change can be measured

an increase in the rotational period suggests bacterial growth/proliferation whereas a constant rotational period suggests growth inhibition

this process can be observed directly by illuminating the culture broth (in the form of a hanging drop) with a light-emitting diode or laser - the hanging-drop format also acts as a lens to create magnification of up to 100 x such that the structure and rotational rate of the bead aggregates can be observed microscopically or when projected onto a detector

revolution ?

non-microorganism detection based

molecular diagnostic approaches

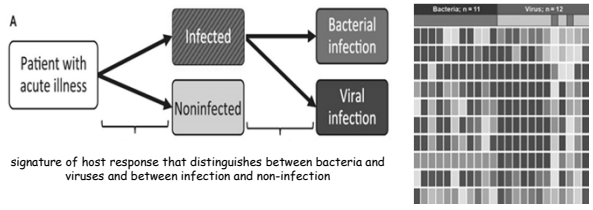
(host response diagnostics)

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)



signature of host response that distinguishes between bacteria and viruses and between infection and non-infection

identification of several expression-based "classifiers"

↓

reduction of expression-based "classifiers"

↓

validation on well-characterized cohorts

↓

real-life validation

low-density arrays

↓

FilmArray/Xpert style real-time assays

↓

point-of-care tests

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

2-transcript signature (FAM89A and IFI44L)

23/23 patients with microbiologically confirmed bacterial infection classified as bacterial (sensitivity, 100% [95%CI, 100%-100%])

27/28 patients with definite viral infection classified as viral (specificity, 96.4% [95%CI, 89.3%-100%])

Host Gene Expression in Nose and Blood for the Diagnosis of Viral Respiratory Infection

J Infect Dis 2019;219:1151-1161

Jinsheng Yu,[1] Derick R. Peterson,[4] Andrea M. Baran,[4] Soumyaroop Bhattacharya,[5] Todd N. Wylie,[2,3] Ann R. Falsey,[4] Thomas J. Mariani,[5] and Gregory A. Storch[2]

nasal gene expression signal = epithelial cells + variable leukocyte contribution

nasal gene expression signatures good or better for discriminating between children with symptomatic RSV infection, symptomatic non-RSV, asymptomatic, and uninfected children

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
sensitivity: 82.9% (68.6 to 94.3)
specificity: 83.6% (74.6 to 92.7) for the diagnosis of culture-confirmed tuberculosis

Xpert MTB/RIF
sensitivity: 54.3% (37.1 to 68.6)
specificity: 100% (100.0 to 100.0) for the diagnosis of culture-confirmed tuberculosis

A Novel, 5-Transcript, Whole-blood Gene-expression Signature for Tuberculosis Screening Among People Living With Human Immunodeficiency Virus

Clin Infect Dis 2019;69: 77-83

Jayant V. Rajan,[1] Fred C. Semitala,[2] Tejas Mehta,[1] Mark Seielstad,[1] Lani Montalvo,[1] Alfred Andama,[2] Lucy Asege,[2] Martha Nakaye,[2] Jane Katende,[2] Sandra Mwebe,[2] Moses R. Kamya,[2] Christina Yoon,[3] and Adithya Cattamanchi[3]

94% sensitivity and 75% specificity

A 20-Gene Set Predictive of Progression to Severe Dengue

Cell Reports 2019;26:1104-11

Makeda Robinson,[1,2,10] Timothy E. Sweeney,[3,4,9,10] Rina Barouch-Bentov,[1] Malaya Kumar Sahoo,[3] Larry Kalesinskas,[3,4] Francesco Vallania,[3,4] Ana María Sanz,[6] Eliana Ortiz-Lasso,[7] Ludwig Luis Albornoz,[7] Fernando Rosso,[6,8] Jose G. Montoya,[1] Benjamin A. Pinsky,[1,5] Purvesh Khatri,[3,4,11,*] and Shirit Einav[1,2,11,12,*]

three retrospective and one prospective dengue datasets

100% sensitivity and 76% specificity for severe dengue

generalizable across ages, host genetic factors, and virus strains

Diagnosing and Managing Sepsis by Probing the Host Response to Infection: Advances, Opportunities, and Challenges

J Clin Microbiol 2019;57:e00425-19

Ian L. Gunsolus,[a] Timothy E. Sweeney,[b] Oliver Liesenfeld,[b] Nathan A. Ledeboer[a]

two transcriptomic sepsis scores validated in independent cohorts using locked algorithm

SeptiScore – FDA cleared - 4 mRNA based test - SeptiCyte Lab (Immunexpress, Seattle, WA)

Sepsis MetaScore – test based on expression levels of 11 host mRNAs

revolution ?

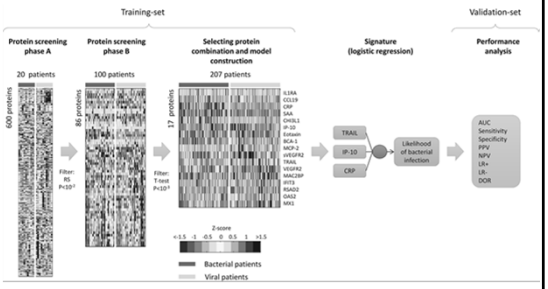
non-microorganism detection based

non-molecular diagnostic approaches

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[1,4], Ellen Bamberger[1,3,5], Yura Fonar[1,6], Renata Yacobov[4], Ron Wolchinsky[4], Galit Denkberg[7], Yaniv Dotan[3,8], Amit Hochberg[9], Yoram Reiter[9], Moti Grupper[3,8], Isaac Srugo[3,8], Paul Feigin[10], Malka Gorfine[10], Irina Chistyakov[3,5], Ron Dagan[11], Adi Klein[4], Israel Potasman[3,5], 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.

Classifier based on 8 short peptide only!

Nasopharyngeal Protein Biomarkers of Acute Respiratory Virus Infection

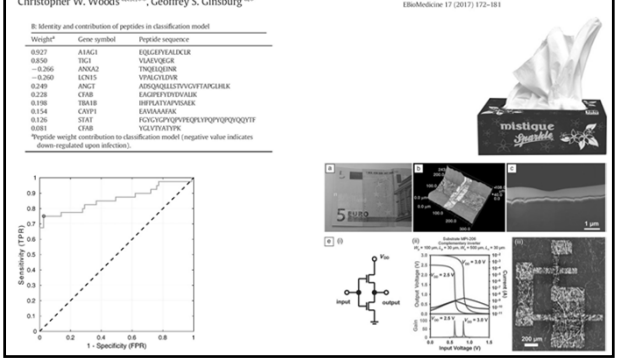
Thomas W. Burke [a], Ricardo Henao [a,d], Erik Soderblom [f], Ephraim L. Tsalik [a,b,c], J. Will Thompson [f], Micah T. McClain [a,c,e], Marshall Nichols [a], Bradly P. Nicholson [b], Timothy Veldman [a], Joseph E. Lucas [a,d], M. Arthur Moseley [d,f], Ronald B. Turner [g], Robert Lambkin-Williams [h], Alfred O. Hero III [i], Christopher W. Woods [a,c,e,*,*], Geoffrey S. Ginsburg [a,*]

EBioMedicine 17 (2017) 172–181

B: Identity and contribution of peptides in classification model

Weight*	Gene symbol	Peptide sequence
0.927	A1AG1	EQLGEFYEALDCLR
0.850	TIG1	VLAEVQEGR
−0.266	ANXA2	TNQELQEINR
−0.260	LCN15	VPALGGYLDVR
0.249	ANGT	ADSQNQLLSTVVGVFTAPGLHLK
0.228	CFAB	EAGIPEFYDYDVALRK
0.198	TBA1B	IHFPLATYAPVISAEK
0.154	CAYP1	EAVIAAAFAK
0.126	STAT	FGYGYGPVQPVPEQPLYPQPYQPQYQQYTF
0.081	CFAB	YGLVTYATYPK

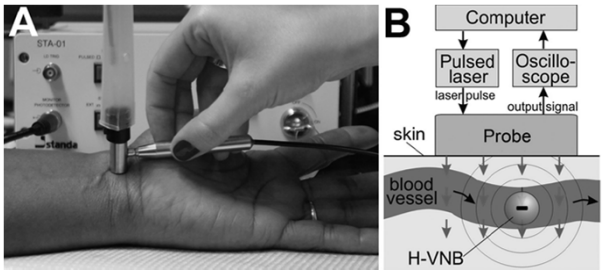
*Peptide weight contribution to classification model (negative value indicates down-regulated upon infection).



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

Narrative Review

Sniffing animals as a diagnostic tool in infectious diseases

Emmanuelle Cambau,[1,2] Mario Poljak[3]

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

J Clin Microbiol 2012; 50: 274-280

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

rats vs. confirmed cases of TB:

sensitivity: 80.4%

specificity: 72.4%

positive predictive value: 41.7%

negative predictive value: 93.8%

REAL LIFE EXPERIENCE

- Belgian non-profit organisation APOPO, which uses rats to detect landmines
- large-scale tuberculosis research programs in Tanzania, Mozambique and Ethiopia
- from 2007 to 2018 more than 550,000 sputum samples were screened by APOPO rats: 14,000 tuberculosis patients detected in addition to standard methods

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Detection of Bacteriuria by Canine Olfaction

Maureen Maurer,[1] Michael McCulloch,[2] Angel M. Willey,[3] Wendi Hirsch,[3] and Danielle Dewey[1] Open Forum Infect Dis 2016; 9:ofw051.

[1]Assistance Dogs of Hawaii, Makawao, Hawaii; [2]Pine Street Foundation, San Anselmo, California; [3]Kapiolani Medical Center for Women and Children, Honolulu, Hawaii

687 individuals; 34% culture-positive and 66% culture-negative controls

dogs detected urine samples positive for 100.000 colony-forming units/mL:

Escherichia coli (N = 250 trials) = sensitivity 99.6%, specificity 91.5%
Enterococcus (n = 50) = sensitivity 100%, specificity 93.9%
Klebsiella (n = 50) = sensitivity 100%, specificity 95.1%
Staphylococcus aureus (n = 50) = sensitivity 100%, specificity 96.3%

Using Dog Scent Detection as a Point-of-Care Tool to Identify Toxigenic *Clostridium difficile* in Stool

Maureen T. Taylor,[1,2] Janine McCready,[1,2] George Broukhanski,[3,4] Sakshi Kirpalaney,[5] Haydon Lutz,[1] and Jeff Powis[1,2] Open Forum Infect Dis 2018;5:ofy179

operating characteristics of 2 comparably trained dogs as a "point-of-care" diagnostic tool to detect toxin gene-positive *Clostridium difficile*

aalthough each dog could detect toxin gene-positive *C. difficile* in stool specimens with sensitivities of 77.6 and 92.6 and specificities of 85.1 and 84.5, interrater reliability is only modest (Cohen's kappa 0.52), limiting widespread application

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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

volatile organic compounds (VOCs)

thermal desorption-gas chromatography mass spectrometry

prospectively collected breath samples

patients with proven or probable pulmonary aspergillosis vs. patients without aspergillosis

94% sensitivity (95% CI, 81%-98%)

93% specificity (95% CI, 79%-98%)