Diagnostik multiresistenter Erreger

Multiplex PCR, Whole Pathogensequencing und andere – Was ist praxisrelevant?

Prof. Dr. med. Karsten Becker
Friedrich Loeffler-Institut
für Medizinische Mikrobiologie
Universitätsmedizin Greifswald

Paul-Ehrlich-Gesellschaft
für Chemotherapie e.V.
www.p-e-g.org

PEG Jahrestagung
digital; 7.-9.10.2021

Empfindlichkeitsbestimmung (Resistenztestung)

determiniert den therapeutischen Einsatz der Antibiotika
(welche und wie)

determiniert die Relevanz der Antibiotikatestung
(ob und welche)

Testarten und Einsatzgebiete*

	Optimierungs-ziel*	Einsatz-zweck	Einsatz-ort	Befund
Standardtest	Sensitivität: ++/+++*** **Spezifität: +++** Schnelligkeit: +	Routine-diagnostik	Labor	**Endbefund**
Screeningtest	**Sensitivität: +++** Spezifität: +/++ Schnelligkeit: +/++	Screening, Epidemiologie	Labor, PoC	Vorläufiger (!) Befund (Klinik)
Schnelltest	Sensitivität: +/++ Spezifität: +/++ **Schnelligkeit: +++**	Akut-situation	Labor, PoC	Vorläufiger (!) Befund
Point-of-care (PoC)-Test	Sensitivität: +/++ Spezifität: +/++ **Schnelligkeit: ++/+++**	Akut-situation	**PoC**	Vorläufiger (!) Befund

* in der Med. Mikrobiologie (vereinfacht)

** epidemiologisch determinierte **NPV und PPV berücksichtigen**

*** Ausgleich durch den parallelen oder nachgeschalteten Einsatz verschiedener Verfahren

Universitätsmedizin Göttingen Karsten Becker

Testarten und Einsatzgebiete

Sensitivität
Spezifität

Epidemiologie
der jeweiligen Population
(lokal, regional, national, global)

Positive predictive value (PPV)
Negative predictive value (NPV)

DS

(Diagnostic stewardship)

Microbiological Diagnostics to → Improve patient outcomes
→ Strengthen health systems
→ Inform disease control strategies

World Health Organization Special Programme for Research and Training in Tropical Diseases (WHO/TDR)

2003

Set of (ASSURED) criteria for the ideal test (primarily for developing countries)

Countries: Developing

A – Affordable	**x**
S – Sensitive	**x**
S – Specific	**x**
U – User-friendly	**x**
R – Rapid and robust	**x**
E – Equipment-free	**x**
D – Deliverable to end-users	**x**

Mabey et al. *Nat Rev Microbiol* 2004; 2(3): 231-240; Land et al. Nat Microbiol (2019); 4(1): 46-54.

Microbiological Diagnostics to → Improve patient outcomes → Strengthen health systems → Inform disease control strategies

World Health Organization Special Programme for Research and Training in Tropical Diseases (WHO/TDR)

TDR For research on diseases of poverty
UNICEF • UNDP • World Bank • WHO

2003 →

Set of (ASSURED) criteria for the ideal test (primarily for developing countries)

	Countries: Developing	Developed
R – Real-time connectivity	**x**	**(x)?**
E – Ease of specimen collection	**x**	**(x)?**
A – Affordable	**x**	**(x)?**
S – Sensitive	**x**	**x**
S – Specific	**x**	**x**
U – User-friendly	**x**	**x**
R – Rapid and robust	**x**	**x**
E – Equipment-free	**x**	**(x)?**
D – Deliverable to end-users	**x**	**(x)?**

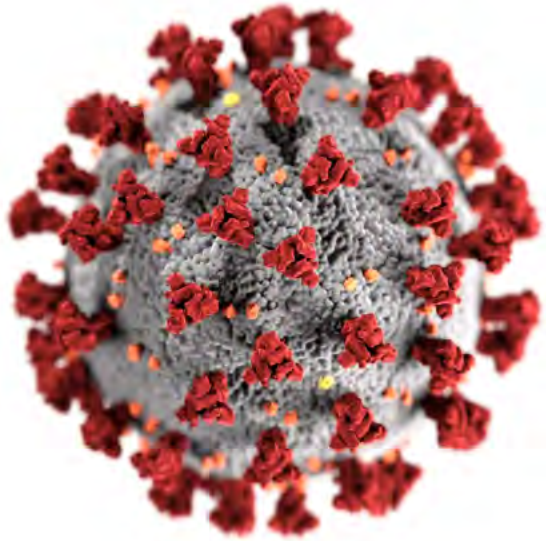
2019 →

Set of (REASSURED) criteria for the ideal test (primarily for developing countries)

Universitätsmedizin Karsten Becker

Mabey et al. *Nat Rev Microbiol* 2004; 2(3): 231-240; Land et al. Nat Microbiol (2019); 4(1): 46-54.

→ COVID-19 pandemics as „game changer“



	Countries: Developing	Developed
R – Real-time connectivity	x	(x)?
E – Ease of specimen collection	x	(x)?
A – Affordable	x	(x)?
S – Sensitive	x	x
S – Specific	x	x
U – User-friendly	x	x
R – Rapid and robust	x	x
E – Equipment-free	x	(x)?
D – Deliverable to end-users	x	(x)?

https://www.br.de/nachrichten

https://coronaabstrich.med.uni-greifswald.de

https://www.schulministerium.nrw/lolli-tests

www.medizin.uni-greifswald.de

Cartridge-based microfluidics with readout systems

Lateral flow devices

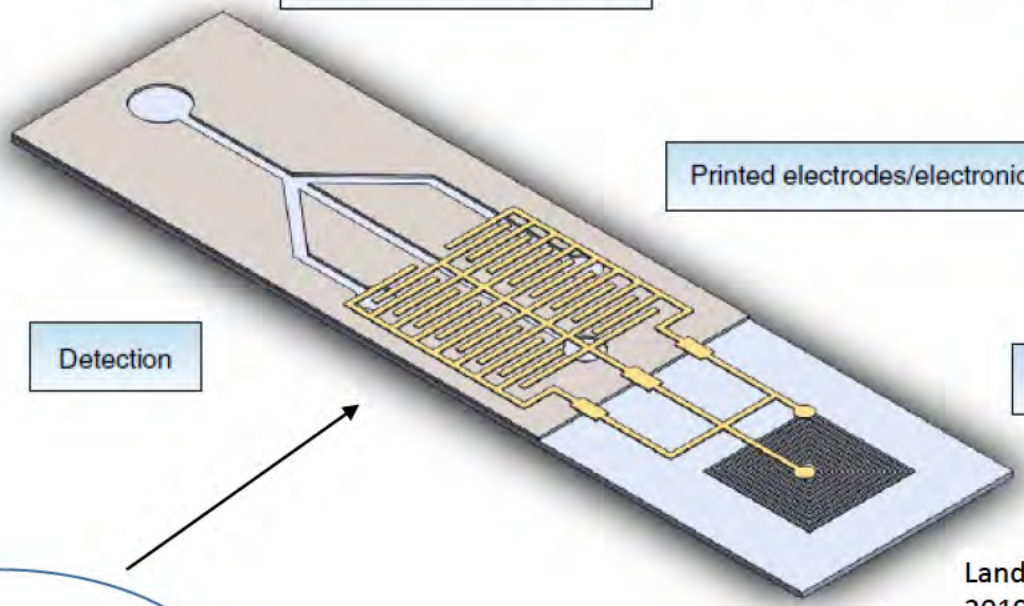
Printed microfluidic channels

Sample introduction

Printed electrodes/electronics

Detection

Printed communication



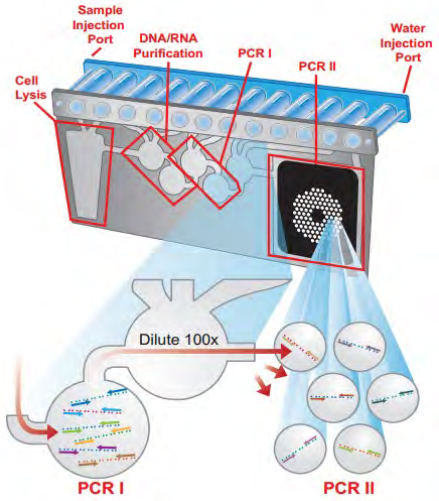
Land et al., Nat. Microbiol. 2019 (mod.)

Molecular and synthetic biology

Internet of Things (Industry 4.0)

Multiplexed PCR systems

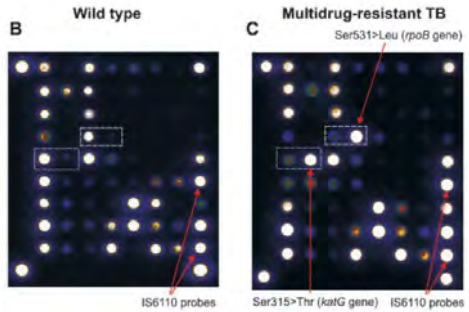
- sehr hohe Sensitivität (Target-Amplifikation!) und hohe Spezifität
- derzeit „**Arbeitspferd**“ für viele diagnostische Anwendungen (Erreger- und Resistenzgen-Nachweise) incl. Quantif.
- viel technologische Erfahrung, technisch relativ „ausgereizt“ (endliche Anzahl an parallel nachgewiesenen Targets)
- preiswert, meist leicht an Gerätepark adaptierbar
- oft externe Qualitätskontrollen („Ringversuche“) verfügbar
- mäßig schnell (4-8 h)



BIOFIRE® FILMARRAY®
www.biomerieux-diagnostics.com

Microarrays (solid-phase hybridization)

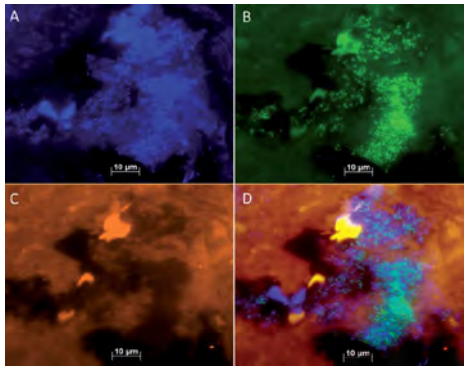
- geringere Sensitivität (<u>keine</u> Target-Amplifikation; gut bei natürlich „vor-amplifizierten“ Erregern in pos. Blutkulturen), z.T. Spezifitätsprobleme)
- propagiert als **„Syndrom“diagnostik** (Vorteil: sehr hohe Multiplexizität mögl., oft incl. viraler Erreger; Nachteil: keine Quantif.)
- sehr teuer, in house oder gerätegebunden
- keine/kaum externe Qualitätskontrollen
- schnell (2-4 h)



Mikhailovich et al., BioEssays 2008

Fluorescence in situ hybridization (FISH)

- Sensitivität?, Spezifität (Vorteil: Lokalisationskontrolle)? (überschaubare Datenlage für routinediagnostische Anwendungen)
- für Spezialfragestellungen: Biofilm-Untersuchung (auch lebend/tot)
- bisher überwiegend in house-Verfahren (Erfahrung!)
- keine Standardisierung, bisher keine externen Qualitätskontrollen
- sehr schnell (0,5-2 h)

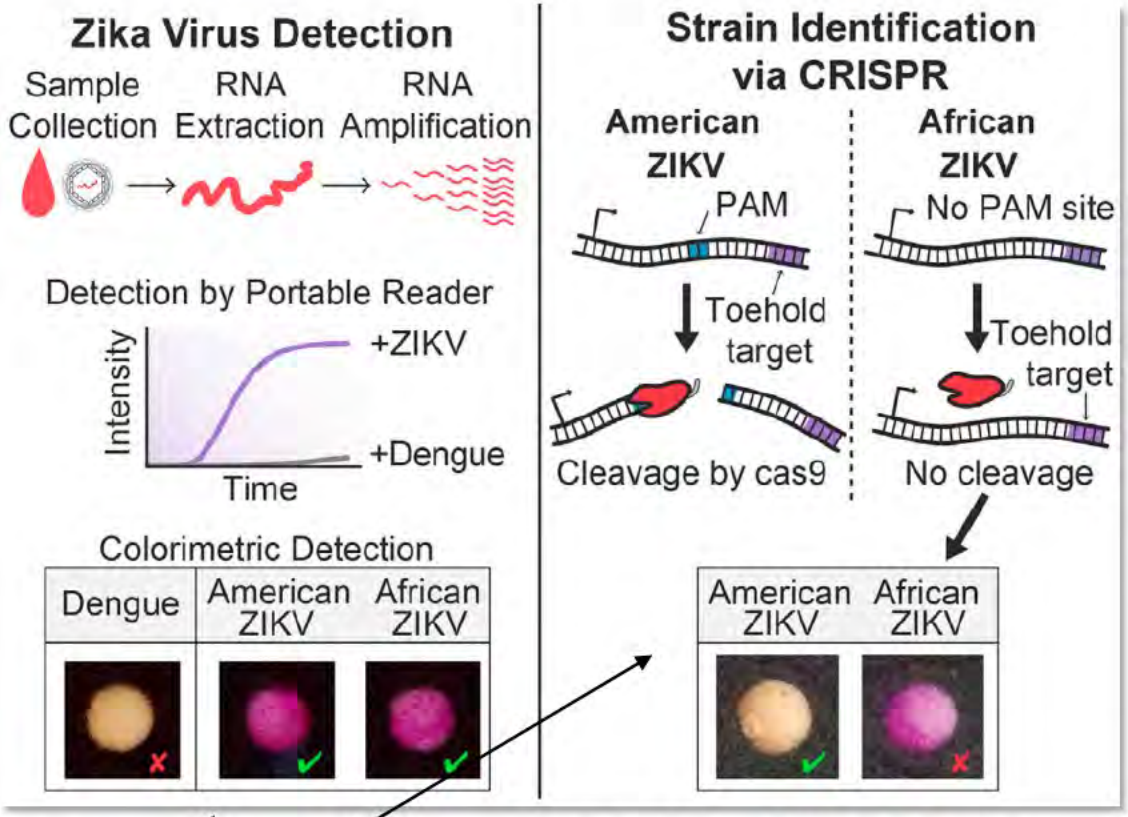


Frickmann et al., *Crit Rev Microbiol* 2017

Pardee et al., 2016, Cell 165, 1255–1266

Cell

Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components



Molecular and synthetic biology

Principle: Ability of Cas9 to selectively cleave DNA only in the presence of an NGG protospacer adjacent motif (PAM) which is strain-specific

Linking:

- Isothermal RNA amplification

+

- Toehold switch RNA sensors (= programmable synthetic riboregulators)

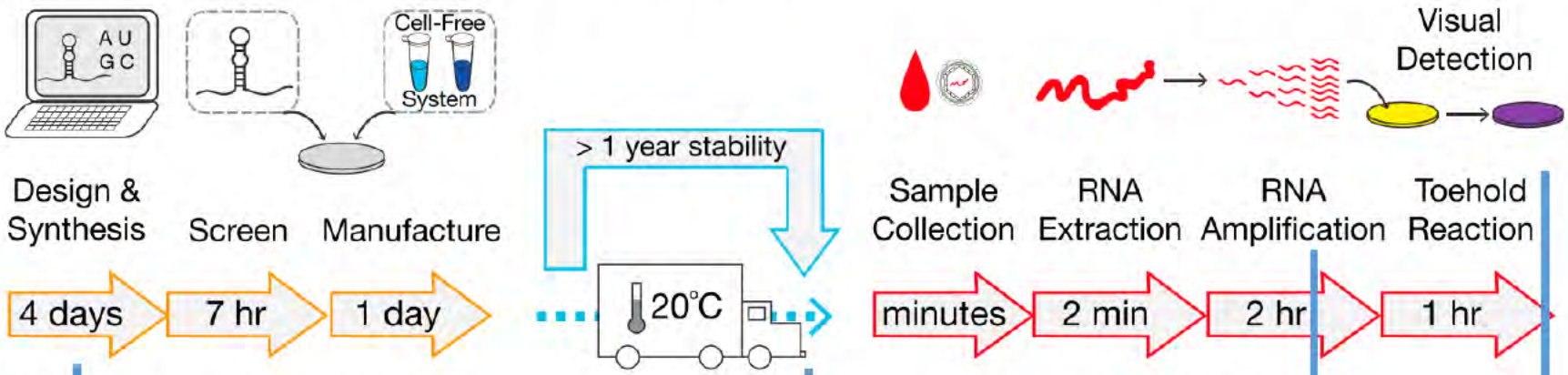
Discrimination with single-base resolution

Universitätsmedizin Karsten Becker

Cell

Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components

Pardee et al., 2016, Cell 165, 1255–1266



Freeze-dried biomolecular platform

in silico-design:

- Primers for isothermal RNA amplification
- Toehold switch-based RNA sensors

Isothermally amplified (NASBA)

Detection of the appropriate trigger RNA by color change in the paper disc

Synthetic biology pipeline for rapid sensor design and prototyping

NASBA-CRISPR Cleavage (NASBACC)

Universitätsmedizin Karsten Becker

PCR

Vermeidung Temperatur-abhängiger Zyklen

isothermale Verfahren

(**NASBA**, **LAMP**, HAD, MDA, RCA, RPA)

Überwindung von:

- geringerer Sensitivität und Spezifität
- komplizierten Reaktionsabläufen
- hohen Kosten
- z.T. fehlender Multiplex-Fähigkeit

Diagnostische CRISPR-Plattformen

- **höherer Spezifitätslevel** (spezifische Primer + Cas-Komplex-Spezifität)
- **Erkennung natürlich variabler Sequenzen** (Mismatch-Bindung durch CRISPR-Proteine)
- hohe Komponenten-Stabilität
- keine vorhergehende Nukleinsäure-Extraktion (bei den neuesten Systemen)

NASBA, nucleic acid sequence-based amplification; LAMP, loop-mediated isothermal amplification; HDA, helicase-dependent amplification; MDA, multiple displacement amplification; RCA, rolling circle amplification; RPA, recombinase polymerase amplification

CRISPR-Cas systems for diagnosing infectious diseases

Tuberculosis	DNA, antibiotic resistance testing	Chimeric dCas9 luciferase (PC Reporter) [54], CRISPR-MTB [106]
HIV	RNA	AIOD-CRISPR [74],
HPV	DNA, virus typing	ctPCR [55], CARP [77], ctPCR3.0 [56], DETECTR, CDetection [79], CRISPR-Cas12a-Mediated Interfacial Cleaving of Hairpin DNA Reporter for Electrochemical Nucleic Acid Sensing [83]
HBV	DNA, genotyping	CRISPR-Cas13a [115]
EBV	DNA, genotyping	SHERLOCK [143]
HBV-HDV co-infection	RNA, genotyping	–
HCV	RNA, genotyping, drug resistance testing	–
Influenza virus	RNA	SHERLOCK [144], CRISPR-Cas13a [145]

Kostyusheva et al. Methods 2021; in press

CRISPR-Cas systems for diagnosing infectious diseases

SARS-CoV	RNA	DETECTR, OR-DETECTR etc.
MERS-CoV	RNA	OR-DETECTR [71]
SARS-CoV-2	RNA	SHERLOCKv2 (FDA approved) [96] DETECTR [71], OR-DETECTR, OR-SHERLOCK, AIOD-CRISPR, CRISPR-Cas12a-NER, SHINE, CRISPR-FDS, iSCAN, CONAN, CASdetec, VaNGuard, STOPCovid, CREST [146], PGMs-CRISPR [85], CARMEN-Cas13[95], CRISPR-LbCas13 [98], opvCRISPR [86], CODA[87], CALIBURN [88]
Dengue virus	RNA	SHERLOCK, SHERLOCKv2, HUDSON + SHERLOCKv2
Ebola virus	RNA	SHERLOCK EBOV assay [147], CRISPR-Cas13a [91]
Zika virus	RNA	SHERLOCK [18], SHERLOCKv2 [70], HUDSON + SHERLOCK [75], NASBACC [17]

Kostyusheva et al. Methods 2021; in press

REASSURED criteria	Countries: Developing	Developed
R – Real-time connectivity	x	(x)
E – Ease of specimen collection	x	(x)
A – Affordable	x	(x)
S – Sensitive	x	x
S – Specific	x	x
U – User-friendly	x	x
R – Rapid and robust	x	x
E – Equipment-free	x	(x)
D – Deliverable to end-users	x	(x)

→ Identifizierung √

→ Antibiotika-Empfindlichkeitstestung ?

Developmental roadmap for antimicrobial susceptibility testing systems

NATURE REVIEWS | MICROBIOLOGY
VOLUME 17 | JANUARY 2019 | 51

CONSENSUS STATEMENT

Alex van Belkum[1], Till T. Bachmann[2], Gerd Lüdke[3], Jan Gorm Lisby[4], Gunnar Kahlmeter[5], Allan Mohess[6], Karsten Becker[7], John P. Hays[8], Neil Woodford[9], Konstantinos Mitsakakis[10], Jacob Moran-Gilad[11,12], Jordi Vila[13], Harald Peter[14], John H. Rex[15] and Wm. Michael Dunne Jr[16], the JPIAMR AMR-RDT Working Group on Antimicrobial Resistance and Rapid Diagnostic Testing*

Abstract | Antimicrobial susceptibility testing (AST) technologies help to accelerate the initiation of targeted antimicrobial therapy for patients with infections and could potentially extend the lifespan of current narrow-spectrum antimicrobials. Although conceptually new and rapid AST technologies have been described, including new phenotyping methods, digital imaging and genomic approaches, there is no single major, or broadly accepted, technological breakthrough that leads the field of rapid AST platform development. This might be owing to several barriers that prevent the timely development and implementation of novel and rapid AST platforms in health-care settings. In this Consensus Statement, we explore such barriers, which include the utility of new methods, the complex process of validating new technology against reference methods beyond the proof-of-concept phase, the legal and regulatory landscapes, costs, the uptake of new tools, reagent stability, optimization of target product profiles, difficulties conducting clinical trials and issues relating to quality and quality control, and present possible solutions.

Zielstellung für AST-Entwicklungen

aber:

van Belkum et al. 2019 *Nat Rev Microbiol* 17: 51-62; Lamy et al. 2020 Clin Microbiol Infect 26:142-150; Rentschler et al. 2021 Int J Mol Sci 22(1); Blondeau & Idelevich 2018 Future Microbiol 13: 1625-1628

Klassische Kultur-basierte Verfahren zur Antibiotika-Empfindlichkeitsprüfung

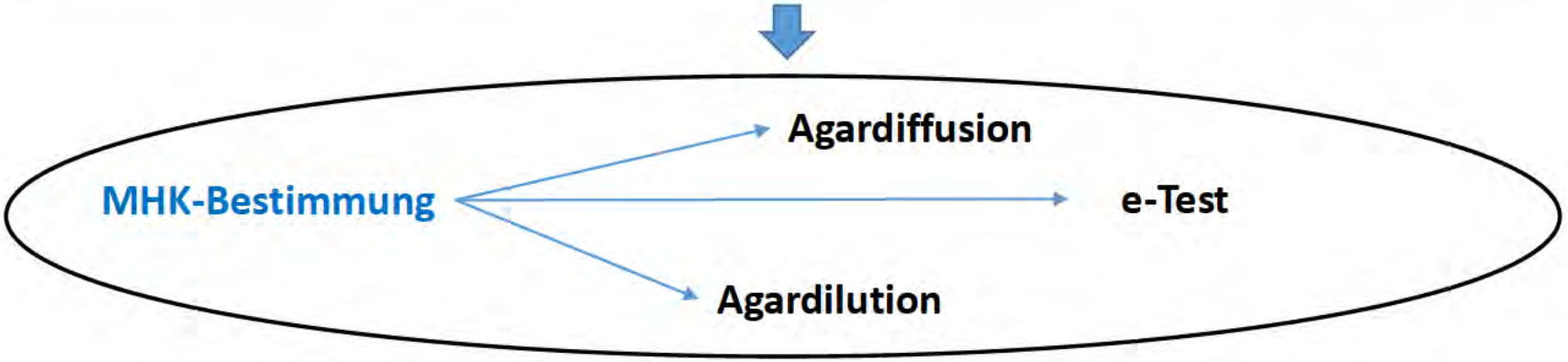
Zur Untersuchung von pathogenen Organismen.[1)]

Von

Dr. R. Koch,
Regierungsrat.

Die Reinkultur ist, wie schon mehrfach betont wurde, für die weitere Ausbildung der Lehre von den pathogenen Organismen und allem, was damit zusammenhängt, ganz unentbehrlich, und in irgendeiner Weise muß Rat geschafft werden, um eine leicht zu handhabende und exakte Methode derselben zu erlangen. Auf dem jetzt eingeschla-

[1)] Aus Mitteilungen aus dem Kaiserl. Gesundheitsamte, Bd. I, 1881, Berlin.



„Übernacht-Bebrütungs“-Mentalität

How to accelerate antimicrobial susceptibility testing

E.A. Idelevich*, K. Becker

Idelevich & Becker 2019; Clin Microbiol Infect 25(11): 1347-1355.

Routine antimicrobial susceptibility testing (AST)

Time to result

Vitek 2
appr. 7 – 18 h

Phoenix
appr. 8 – 18 h

Walkaway
appr. 12 – 48 h

Sensititre
appr. 12 – 24 h

- **Phenotypic approach!**
- Broad panel of antibiotic agents
- EUCAST/CLSI-compliant
- Final result

- **Slow („overnight“)!**
- Phenotypic approach
- Broad panel of antibiotic agents

Idelevich & Becker 2019; *Clin Microbiol Infect* 25(11): 1347-1355.

Universitätsmedizin Karsten Becker

Screening for ESBL and carbapenemases in *E. coli* and *K. pneumoniae* for epidemiological purposes as part of the RAST procedure.

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

EUCAST Guidelines for detection of resistance mechanisms and specific resistance of clinical and/or epidemiological importance using EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles.

Version 1.0

May 2019

Rapid Antimicrobial Susceptibility Testing

limited to selected bacteria-/agent-combinations

Table 1. Screening for ESBL-producing *E. coli* and *K. pneumoniae*.

Species	Antimicrobial agent	Conduct ESBL testing if		
		4 hours	6 hours	8 hours
E. coli	Cefotaxime 5 µg	<15	<16	<17
	Ceftazidime 10 µg	<15	<16	<17
K. pneumoniae	Cefotaxime 5 µg	<15	<18	<18
	Ceftazidime 10 µg	<15	<16	<16

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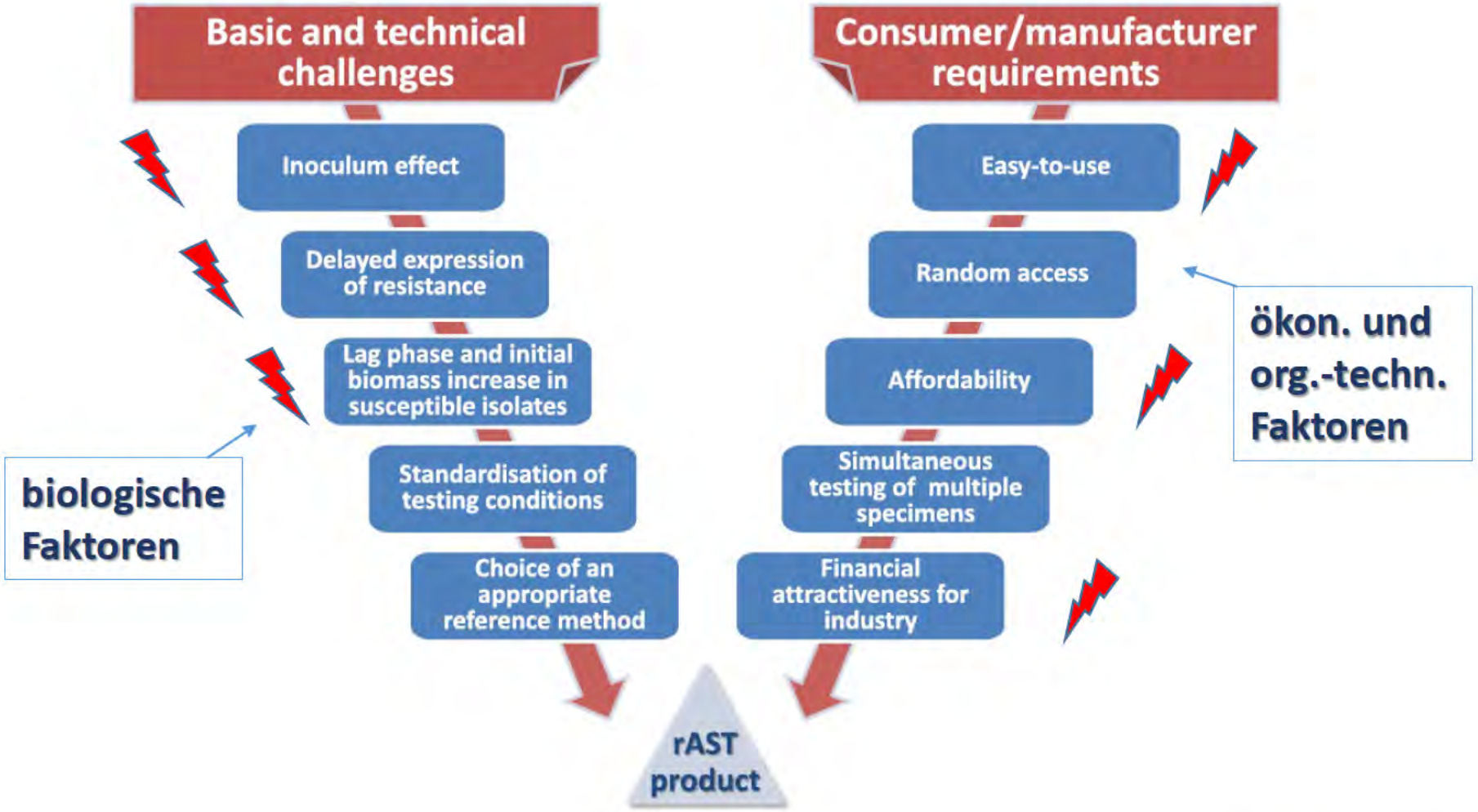
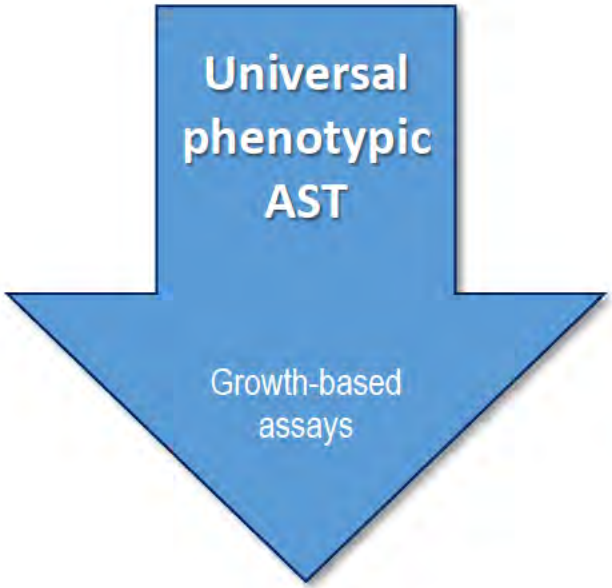
Herausforderungen und Erfordernisse für "rapid antimicrobial susceptibility testing" (rAST)



Idelevich & Becker 2019; *Clin Microbiol Infect* 25(11): 1347-1355.

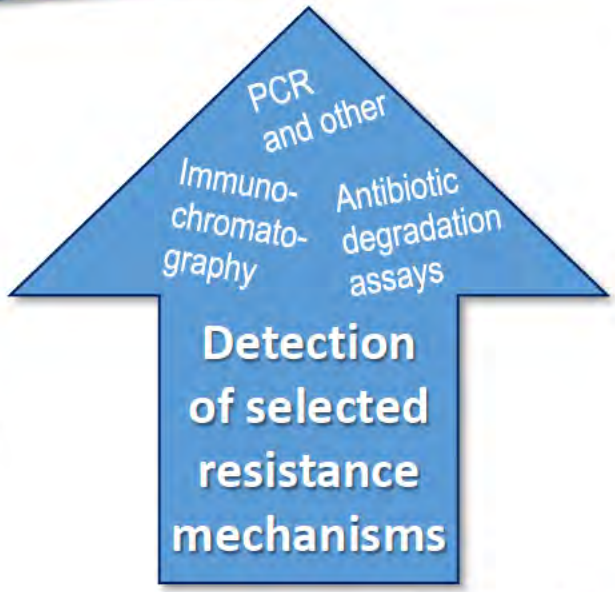


→ Universally applicable
→ Mechanism-independent
→ Exact phenotypic categorisation
→ Therapeutic relevance

→ Time for microbial growth
→ Expression-dependent

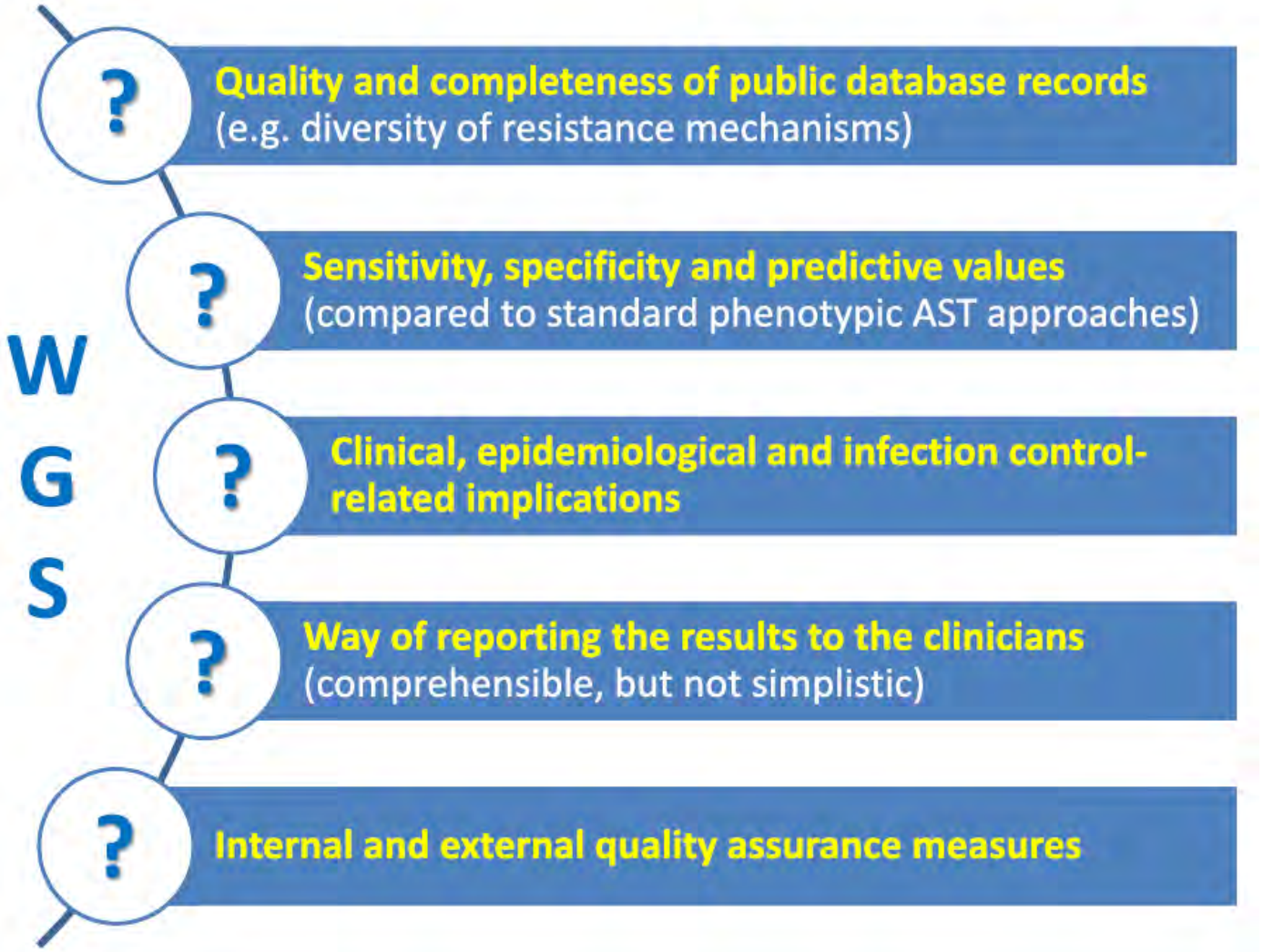
→ Very rapid (approx. 15 min – 1 hour)
→ Confirmation of resistance mechanism

→ Negativity does not necessarily mean susceptibility
→ Positivity does not necessarily confer phenotypic resistance
→ Limited to some antibiotics



Idelevich & Becker 2019; *Clin Microbiol Infect* 25(11): 1347-1355.

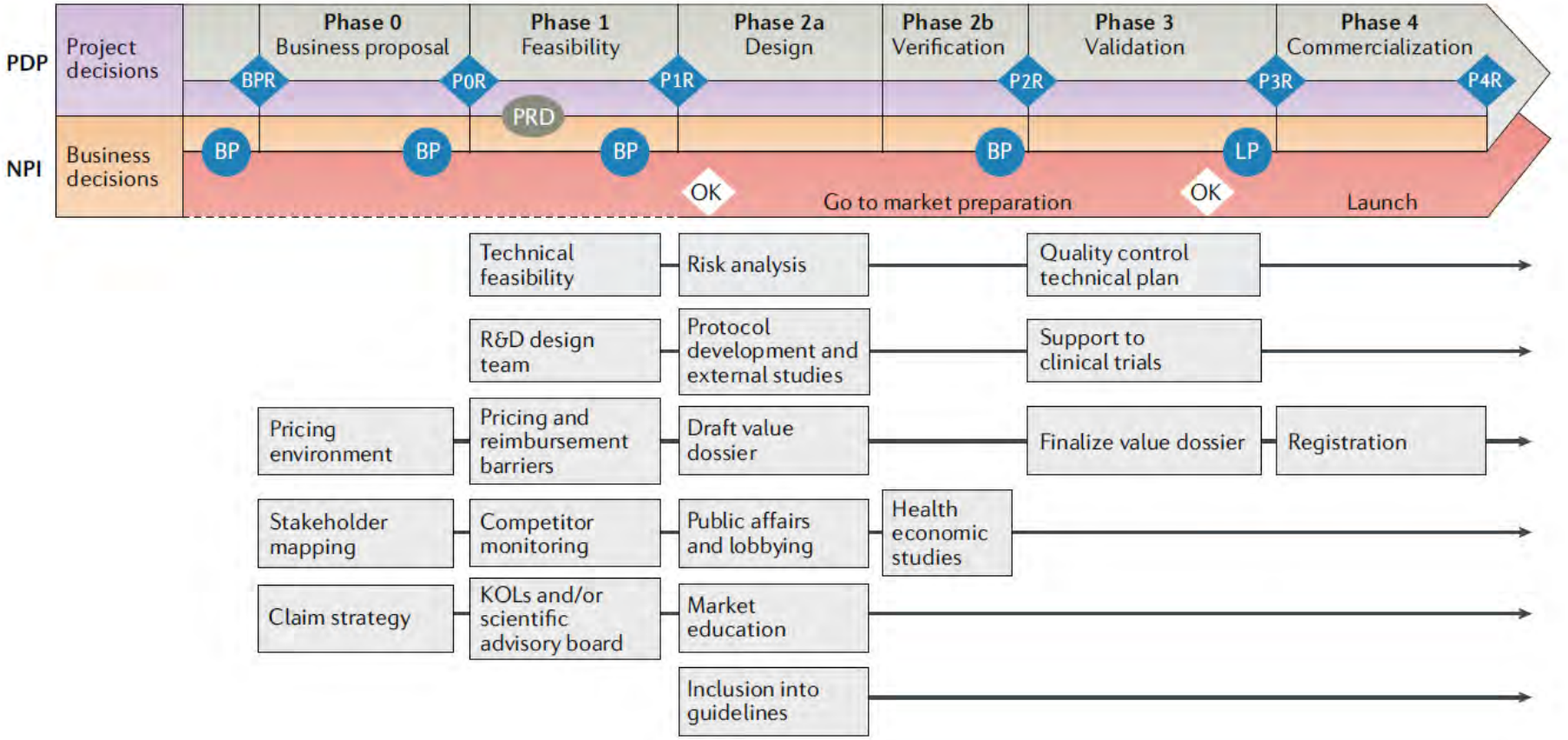
Ungelöste Fragen, die einer Einführung von “whole genome sequencing (WGS)” als AST-Routineanwendung im Wege stehen



Idelevich & Becker 2019; *Clin Microbiol Infect* 25(11): 1347-1355.

It´s a long, long way to product launch...

(except under Covid-19 exceptional conditions)



PDP, product development plan; BP(R), business plan (review); NPI, new product introduction; LP, launch planning

van Belkum et al. 2019; *Nat Rev Microbiol* 17(1): 51-62.

Innovative Verfahren sind nicht per se erfolgreich am Markt...

Polymerase chain reaction (PCR) / electrospray ionization-mass spectrometry (PCR/ESI-MS)

→ 2 Jahre Marktverfügbarkeit in Europa

↓

Routine-Implementierung in nur 3 europ. Krankenhäusern

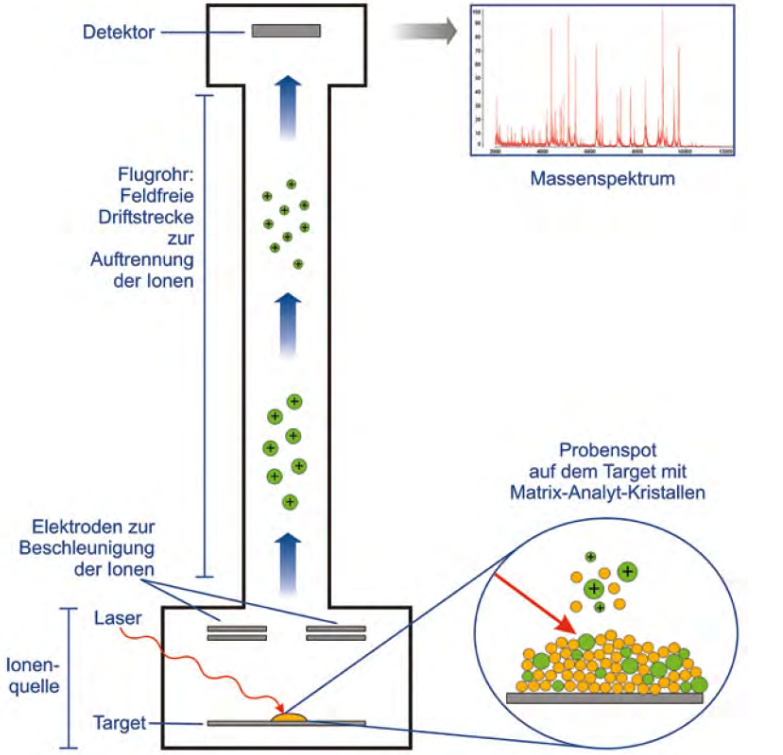
(Systemkosten: ~$300,000 US$
Testkosten: 200–300 US$ (nicht (EBM) oder nur teilweise (GOÄ) rückerstattungsfähig)
Platzbedarf: ~30 m² über 2 Lab.)

Özenci et al. *Clin Infect Dis* 2018; 66: 452-455.

MALDI-TOF-Massenspektrometrie

www.bruker.com

www.biomerieux-usa.com



Burak & Gehrt, 2010

Identifizierung: √

Resistenztestung: ?

Main approaches to the determination of antimicrobial susceptibility and resistance using MALDI-TOF MS

Determination of **changes of the antimicrobial compound** caused by the microbial resistance mechanism

Determination of **specific peaks in microbial spectrum** for particular resistance mechanism

Microbial growth analysis in the presence of antimicrobials

Mechanism-/agent-specific approach

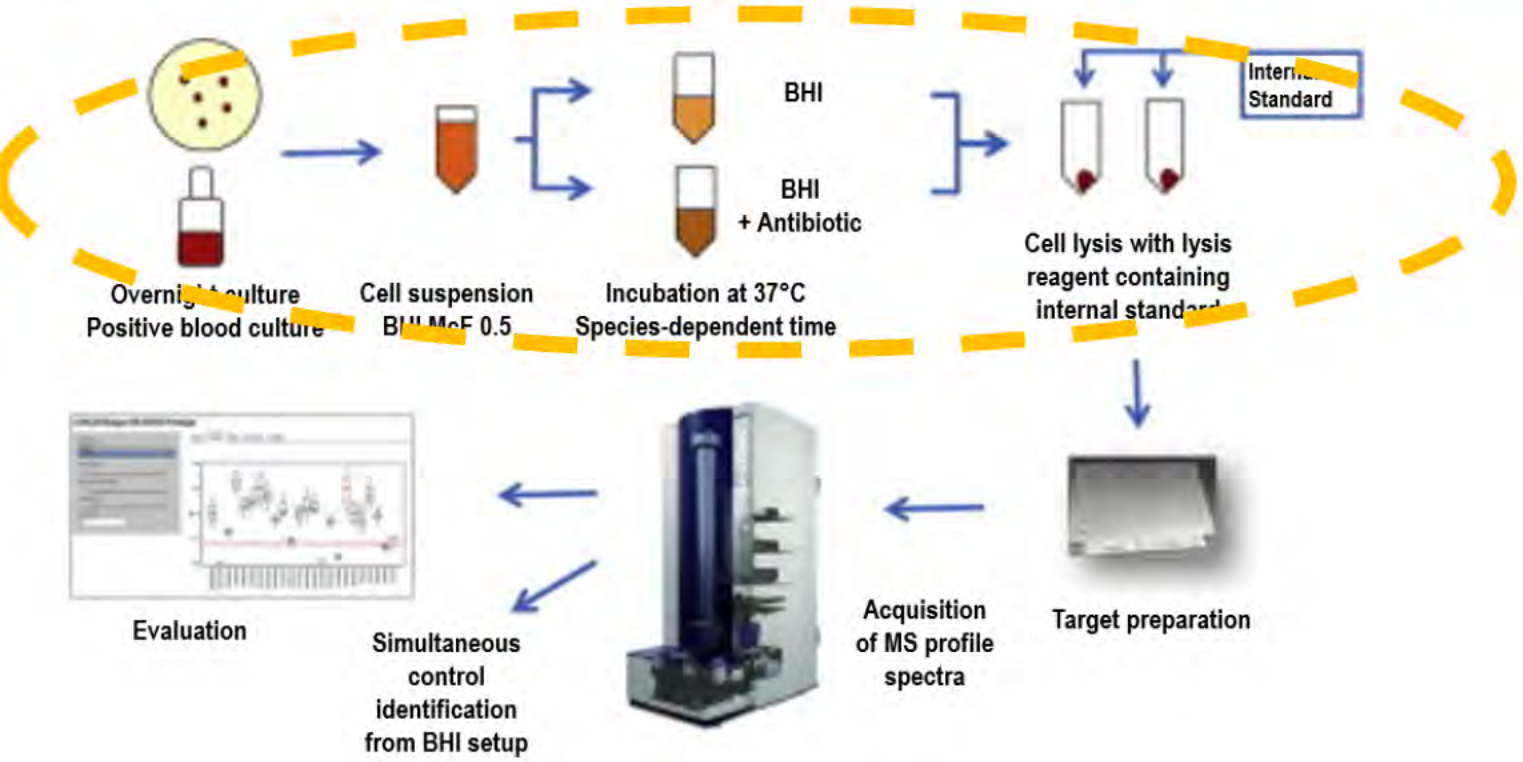
Universal approach

Idelevich & Becker
J Clin Microbiol: JCM0181419 (in press)

MBT-ASTRA: A suitable tool for fast antibiotic susceptibility testing?

Katrin Sparbier [a], Sören Schubert [b], Markus Kostrzewa [a,*]

Methods 104 (2016) 48–54



universelle Antibiotikatestung

separate Inkubation der Erregerflüssigkulturen

Direct-on-Target Microdroplet Growth Assay (DOT-MGA)*

Bacterial suspension in CA-MHB

Antibiotic-containing CA-MHB

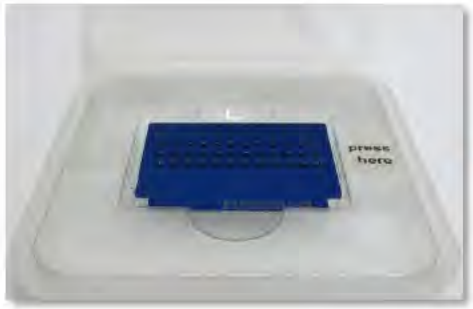
Final inoculum: $5x10^5$ cfu/ml
Breakpoint conc. (meropenem: 2 mg/L)

Growth detection by MALDI-TOF MS

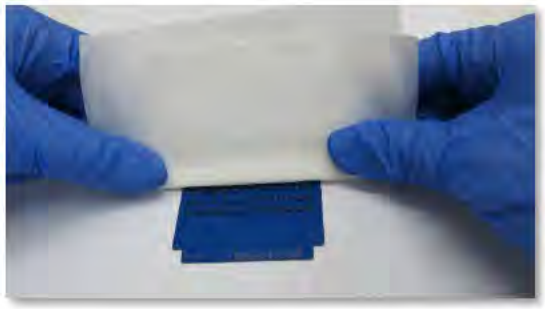
Prototype software for quantitative MALDI-TOF MS**

Overlaying with internal protein standard-spiked matrix

Hydrophilic spots (microdroplets) of disposable MALDI-TOF MS targets (MBT Biotargets-96; Bruker)



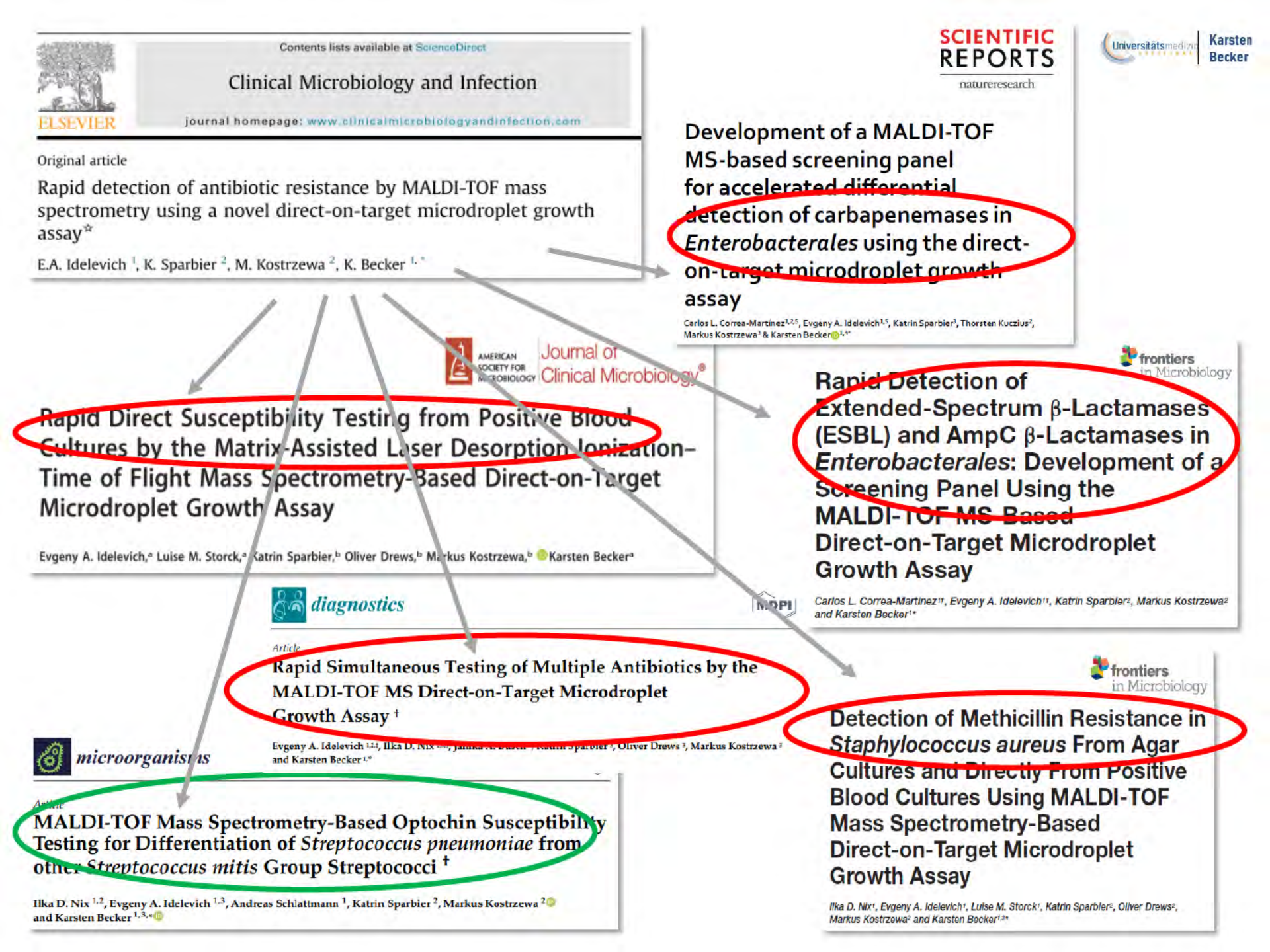
Incubation (36°C) directly on target in a **humidity chamber** (plastic transport box; Bruker)

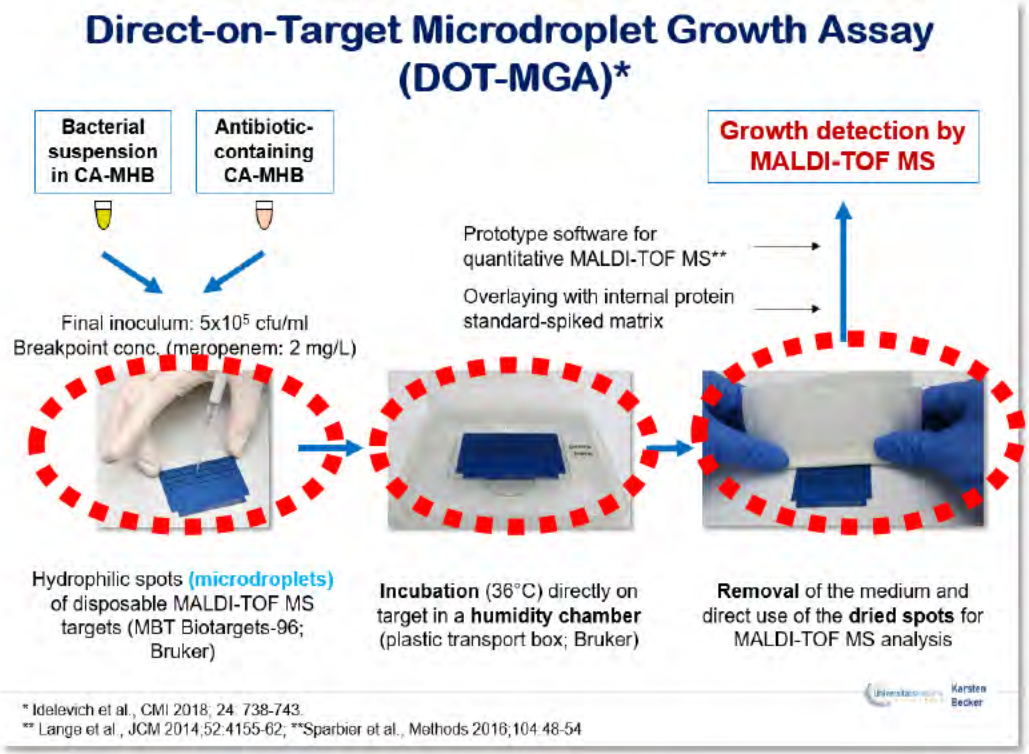


Removal of the medium and direct use of the **dried spots** for MALDI-TOF MS analysis

Universitätsmedizin Greifswald Karsten Becker

* Idelevich et al., CMI 2018; 24: 738-743.
** Lange et al., JCM 2014;52:4155-62; **Sparbier et al., Methods 2016;104:48-54


Contents lists available at ScienceDirect
Clinical Microbiology and Infection
journal homepage: www.clinicalmicrobiologyandinfection.com
Original article
Rapid detection of antibiotic resistance by MALDI-TOF mass spectrometry using a novel direct-on-target microdroplet growth assay*
E.A. Idelevich [1], K. Sparbier [2], M. Kostrzewa [2], K. Becker [1, *]
SCIENTIFIC REPORTS
nature research
Development of a MALDI-TOF MS-based screening panel for accelerated differential detection of carbapenemases in Enterobacterales using the direct-on-target microdroplet growth assay
Carlos L. Correa-Martinez[1,2,5], Evgeny A. Idelevich[1,5], Katrin Sparbier[3], Thorsten Kuczius[2], Markus Kostrzewa[3] & Karsten Becker[1,4*]
Universitätsmedizin
Karsten Becker
AMERICAN SOCIETY FOR MICROBIOLOGY
Journal of Clinical Microbiology
Rapid Direct Susceptibility Testing from Positive Blood Cultures by the Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Direct-on-Target Microdroplet Growth Assay
Evgeny A. Idelevich,[a] Luise M. Storck,[a] Katrin Sparbier,[b] Oliver Drews,[b] Markus Kostrzewa,[b] Karsten Becker[a]
frontiers in Microbiology
Rapid Detection of Extended-Spectrum β-Lactamases (ESBL) and AmpC β-Lactamases in Enterobacterales: Development of a Screening Panel Using the MALDI-TOF MS-Based Direct-on-Target Microdroplet Growth Assay
Carlos L. Correa-Martinez[1†], Evgeny A. Idelevich[1†], Katrin Sparbier[2], Markus Kostrzewa[2] and Karsten Bockor[1*]
diagnostics
MDPI
Article
Rapid Simultaneous Testing of Multiple Antibiotics by the MALDI-TOF MS Direct-on-Target Microdroplet Growth Assay †
Evgeny A. Idelevich [1,2,†], Ilka D. Nix [1,†], Janika A. Bäsch [1], Katrin Sparbier [2], Oliver Drews [3], Markus Kostrzewa [3] and Karsten Becker [1,*]
frontiers in Microbiology
Detection of Methicillin Resistance in Staphylococcus aureus From Agar Cultures and Directly From Positive Blood Cultures Using MALDI-TOF Mass Spectrometry-Based Direct-on-Target Microdroplet Growth Assay
Ilka D. Nix[1†], Evgeny A. Idelevich[1†], Luise M. Storck[1], Katrin Sparbier[2], Oliver Drews[2], Markus Kostrzewa[2] and Karsten Bockor[1,2*]
microorganisms
Article
MALDI-TOF Mass Spectrometry-Based Optochin Susceptibility Testing for Differentiation of Streptococcus pneumoniae from other Streptococcus mitis Group Streptococci †
Ilka D. Nix [1,2], Evgeny A. Idelevich [1,3], Andreas Schlattmann [1], Katrin Sparbier [2], Markus Kostrzewa [2] and Karsten Becker [1,3,*]



Experimental-Stadium

industrielle Konfektionierung/Standardisierung

* Idelevich & Becker, PCT/DE2016/100561

Universitätsmedizin Karsten Becker

Fazit

- keine „eierlegende Wollmilchsau“ für Identifizierung und Empfindlichkeitstestung → **Einsatzzweck beachten und Testlimitationen kennen**
- **vordringliche Entwicklungsaufgabe: Beschleunigung** unter weitgehendem Erhalt der Leistungsparameter (insbes. Sensitivität, Spezifität) und der Anwenderfreundlichkeit (Laborintegration, hands-on-time ...)
- **Beschleunigungsmöglichkeiten auch umsetzen**
  - Reorganisation der Laborprozesse
  - Präanalytik (Transportwege!) optimieren
  - 24/7 (Labor und Klinik, [ÖGD]), Vor-Ort-Mikrobiologie
  - Diagnostic Stewardship (DS)
- Innovationsniveau/Publikationslevel ≠ Marktreife → Marktinteresse → Markt(dauer)erfolg
- Hemmnisse: nur bedingt fortschrittsorientiertes Reimbursement-System (EBM, GOÄ) sowie „neue“ EU-VO In-vitro-Diagnostika (2017)