Rapid Antimicrobial Susceptibility Testing

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IFL Quarterly Webinar
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Disclosures

None

Objectives

- Discuss current rapid AST methods
- Evaluate clinical impact of rapid AST
- Assess future rapid AST technologies

Abbreviations

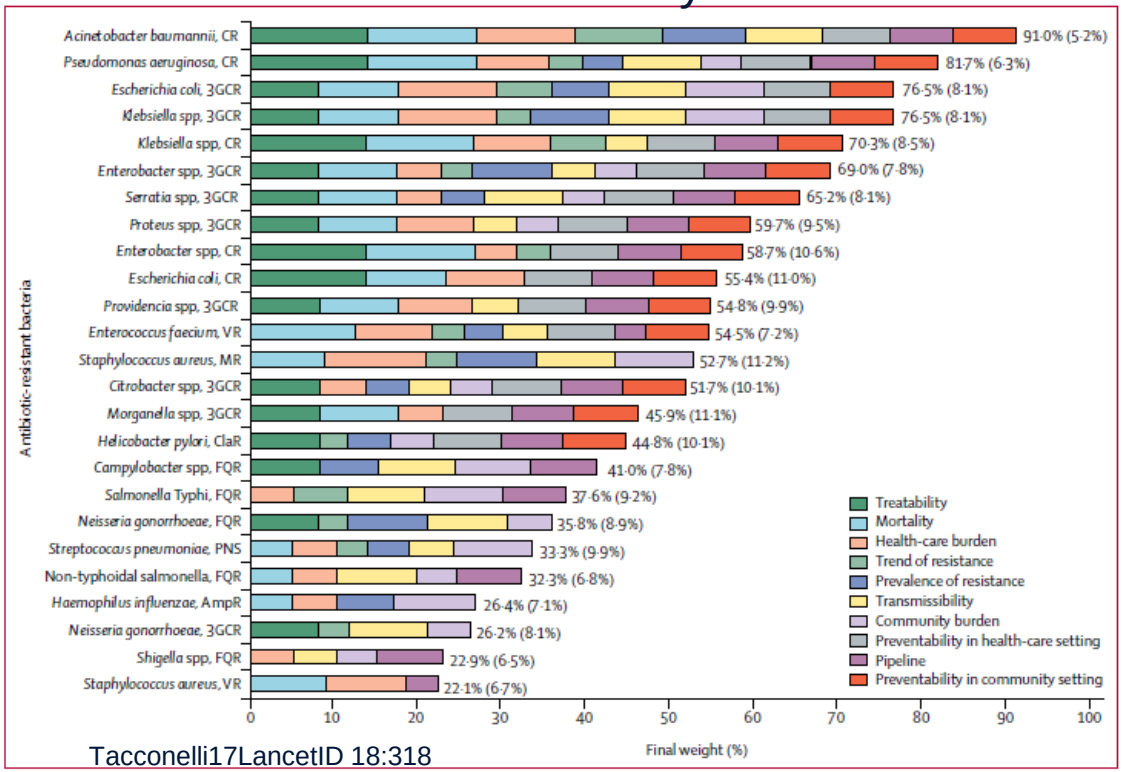
- Abx - antibiotics
- AST – antimicrobial susceptibility testing
- BMD – broth microdilution
- CA – categorical (interpretation) agreement
- DD – disk diffusion (Kirby-Bauer)
- EA – essential agreement (MIC ±1 dilution)
- ID – identification (of organisms)
- LOS – length of stay
- MIC – minimal inhibitory concentration
- TTR – time to results

https://wiki.southpark.cc.com/wiki/Mr._Mackey

Antibiotic resistance

- Increasing concern over antibiotic resistant organisms
- Morbidity and mortality despite a wide array of antibiotics
- Rapid Antimicrobial Susceptibility Testing (AST) should help improve antibiotic use and patient outcomes.

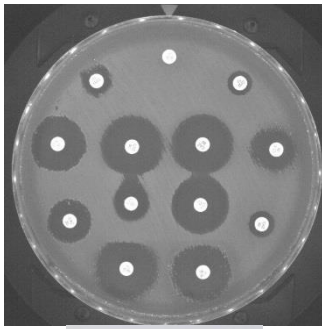
WHO Abx-R Priority List

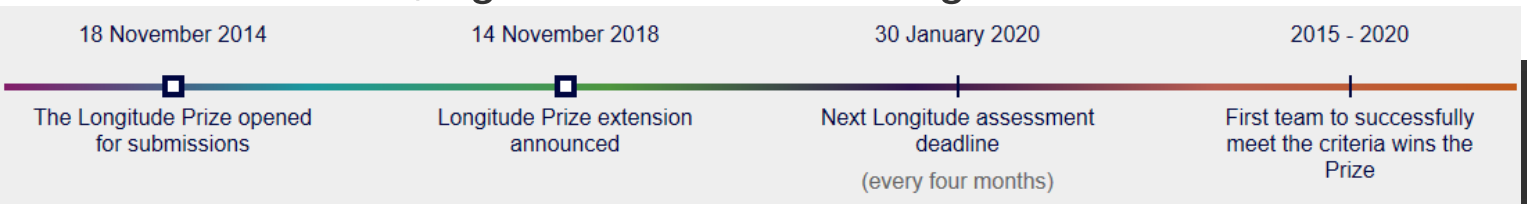


Tacconelli17LancetID 18:318

How rapid is “rapid”?

- Standard reference Antimicrobial Susceptibility Testing (AST) methods require ~18-24h incubation to interpret
  - Not “rapid”
- Are AST results in 12h “rapid”?
  - BD Phoenix AST average time to result (TTR) is ~12h
- 8h?
  - bioMerieux Vitek2 AST average TTR is ~8.5h
- 6h?
  - BD Phoenix AST TTR range is ~6-16h (Microscan G+ similar)
- 4h?
  - bioMerieux Vitek2 AST TTR range is ~4-10h (Microscan G- similar)
- This is as fast as current commercial phenotypic AST gets…
- Current molecular methods can be faster, but don’t give full AST
- **Longitude Prize** (£8 million): <**30min**, POC Dx, usable anywhere, affordable, right antibiotic at the right time





Eigner05JCM 43:3829, longitudeprize.org

Commercial rapid molecular “AST”

- Methicillin resistance in *S. aureus, mecA*
- Vancomycin resistance in *Enterococcus, vanA/B*
- Rifampin resistance in *M. tuberculosis, rpoB*
- Multiplex tests for blood cultures
  - Rapid ID plus limited resistance gene detection: *mecA, vanA/B,* select β-lactamases (common carbapenemases, ± limited ESBL)
- Multiplex test for respiratory specimens
  - Rapid ID plus somewhat broader resistance gene detection: *mecA, vanA/B,* common carbapenemases, limited ESBL, *ermB* (macrolide/lincosamide), *sul1* (sulfonamide), *gyrA* (quinolone)
- Non-FDA-cleared DNA microarrays, multiplex PCRs
  - multiple β-lactamases (AmpC, ESBL, carbapenemases)
- *WGS looks promising, but no commercial AST kits yet*

Molecular “AST” Pros/Cons

- Pros
  - Speed
  - Sensitivity
  - Direct from sample
  - Don’t require pure culture
- Cons
  - Exquisitely targeted (false neg/false susceptible)
  - Detection not directly tied to function (false pos/false resistant)
  - No minimal inhibitory concentration (MIC)
  - Cost
  - Supplemental nature of results (still want “full AST”)

Do clinicians respond to rapid molecular tests?

Clinical Infectious Diseases 2005; 41:1438–44

Impact of Rapid Detection of Viral and Atypical Bacterial Pathogens by Real-Time Polymerase Chain Reaction for Patients with Lower Respiratory Tract Infection

Jan Jelrik Oosterheert,[1] Anton M. van Loon,[2,3] Rob Schuurman,[2,3] Andy I. M. Hoepelman,[1,3] Eelko Hak,[4] Steven Thijsen,[6] George Nossent,[5] Margriet M. E. Schneider,[1] Willem M. N. Hustinx,[7] and Marc J. M. Bonten[1,3,4]

Division of Medicine, [1]Department of Internal Medicine and Infectious Diseases and [2]Department of Virology, [3]Eijkman Winkler Institute for Infectious Diseases, Microbiology, and Inflammation, [4]Julius Center for Health Sciences and Primary Care, and [5]Department of Respiratory Medicine, University Medical Center, and Departments of [6]Medical Microbiology and [7]Internal Medicine, Diakonessenhuis Utrecht, Utrecht, The Netherlands

- No significant difference in mortality, LOS, time on Abx, extra Dx procedures, and increased costs significantly.
- Clinicians hesitant to stop antibiotics based on +viral PCR

Eur J Clin Microbiol Infect Dis (2015) 34:831–838
DOI 10.1007/s10096-014-2299-0

ARTICLE

Impact of same-day antibiotic susceptibility testing on time to appropriate antibiotic treatment of patients with bacteraemia: a randomised controlled trial

J. Beuving · P. F. G. Wolffs · W. L. J. Hansen · E. E. Stobberingh · C. A. Bruggeman · A. Kessels · A. Verbon

- Faster ID and appropriate therapy, but no significant difference in mortality or LOS
- Clinicians hesitant to stop abx based on rapid molecular breakpoint AST (15h faster)

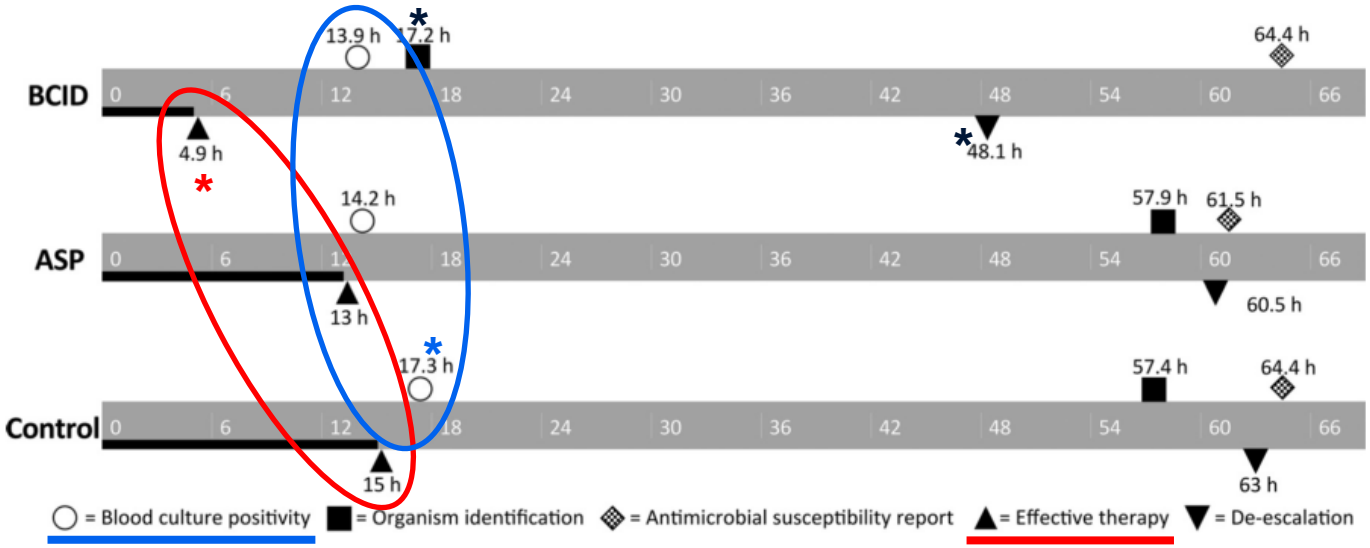
AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Benefits of Adding a Rapid PCR-Based Blood Culture Identification Panel to an Established Antimicrobial Stewardship Program

Shawn H. MacVane,[a,b] Frederick S. Nolte[c]

Department of Pharmacy,[a] Division of Infectious Diseases,[b] and Department of Pathology and Laboratory Medicine,[c] Medical University of South Carolina, Charleston, South Carolina, USA

- Individual contributions of Abx stewardship and rapid ID/"AST"
  - ~100 pts in each intervention. Significantly (40h) faster ID, time to effective therapy.
  - No significant difference pre/post stewardship or BCID for mortality, 30-day readmission, ICU LOS, post-culture LOS, or costs.
  - Noted a "potential hesitancy of providers to narrow the spectrum of antimicrobial activity based on the PCR result alone, prior to [AST] results."



MacVane16JCM 54:2455

Not a new phenomenon

DIAGN MICROBIOL INFECT DIS
1993;16:237–243

The Impact of Same-Day Tests versus Traditional Overnight Testing

Paul A. Granato

"Clinicians appear to have been reluctant to modify initial empiric therapies, however, despite the availability of the rapid antimicrobial susceptibility report."

"There is still an understandable physician reluctance to modify existing therapy to a less expensive, equally efficacious agent in light of a favorable patient response."

- "rapid" in 1993 was not that different than now
  - 9-10h then, 7-8h today

Eur J Clin Microbiol Infect Dis (2005) 24: 305–313
DOI 10.1007/s10096-005-1309-7

ARTICLE

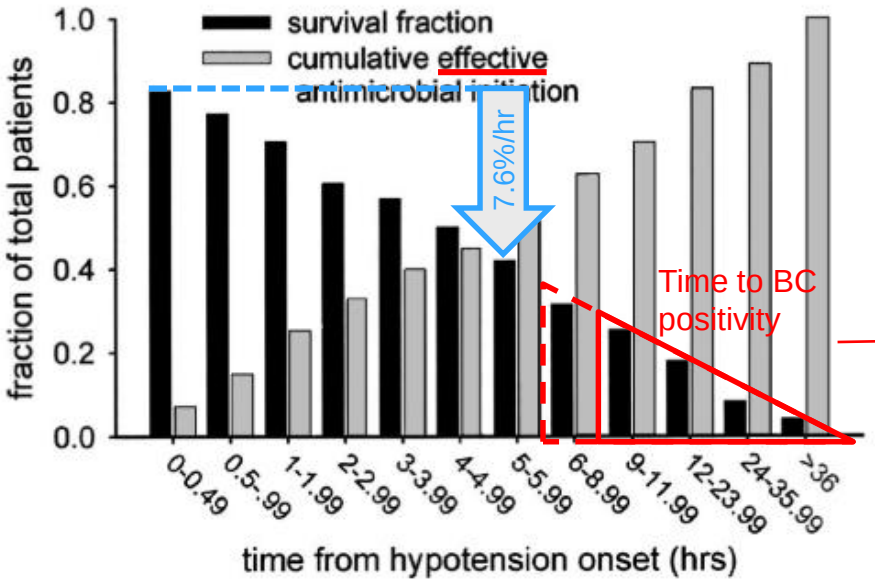
M. Bruins · H. Oord · P. Bloembergen ·
M. Wolfhagen · A. Casparie · J. Degener · G. Ruijs

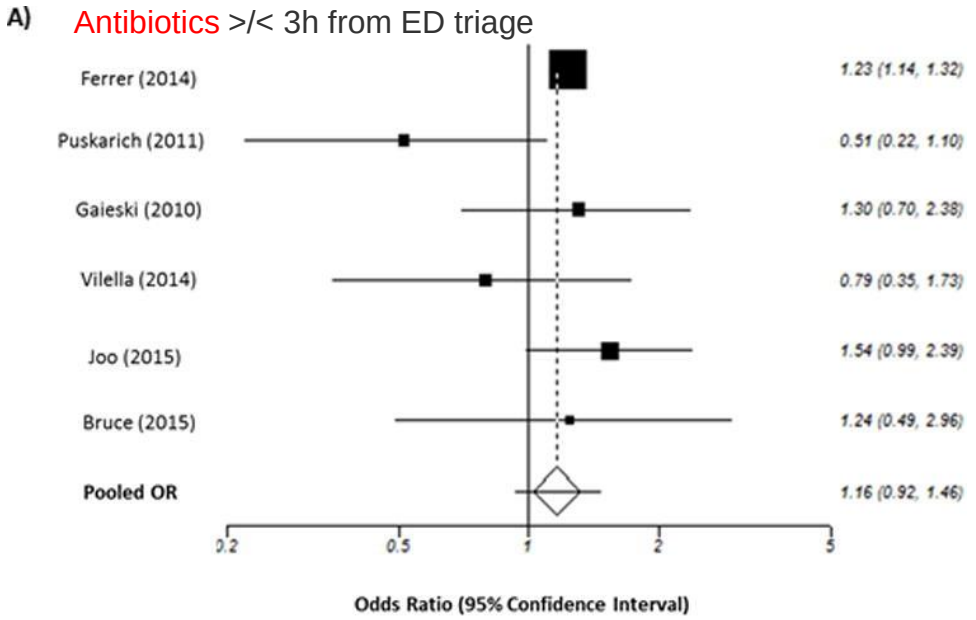
Lack of effect of shorter turnaround time of microbiological procedures on clinical outcomes: a randomised controlled trial among hospitalised patients in the Netherlands

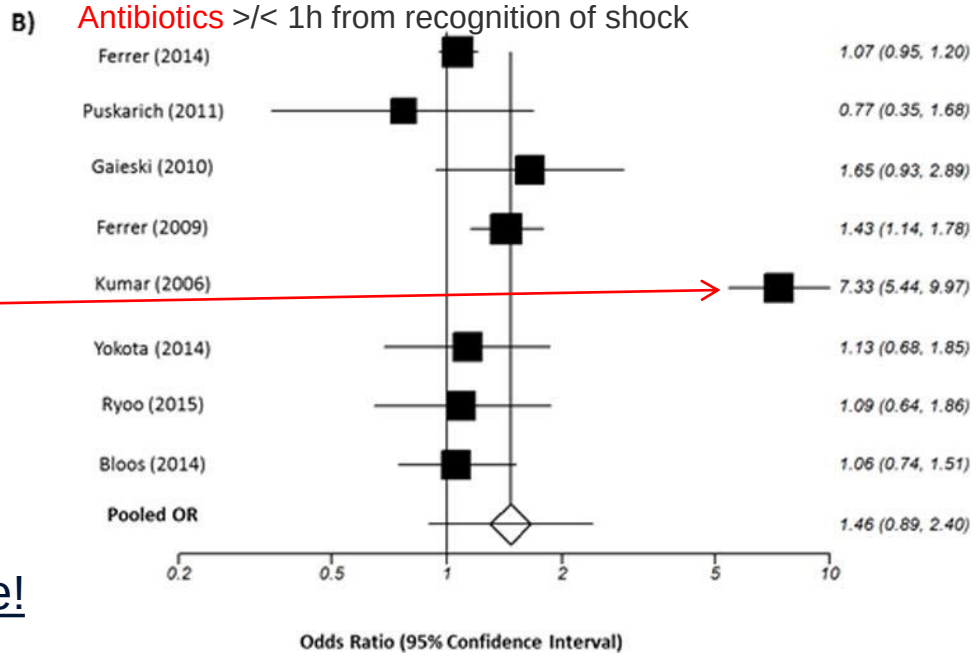
"To affect outcomes significantly, however, efficient clinical follow-up must be ensured, which probably warrants workflow changes in other hospital departments…"

Rapid vs. mortality

- Rapid antibiotics *should* reduce mortality
  - ∴ rapid AST results should also reduce mortality
- Shouldn't they?







Affecting mortality with AST is a challenge!

Kumar06CritCareMed 34:1589

Sterling15CritCareMed 43:1907

So why do we expect better outcomes from rapid AST?

JOURNAL OF CLINICAL MICROBIOLOGY, July 1994, p. 1757–1762 Vol. 32, No. 7
0095-1137/94/$04.00+0

Clinical Impact of Rapid In Vitro Susceptibility Testing and Bacterial Identification

GARY V. DOERN,[1,2]* RAYMOND VAUTOUR,[1] MICHAEL GAUDET,[2] AND BRUCE LEVY[1]

Department of Hospital Laboratories[1] and Division of Infectious Diseases,[2] University of Massachusetts Medical Center, Worcester, Massachusetts 01655

- Prospective, random(ish), all culture types, 300pts/group
- Automated phenotypic AST ~16h faster, ID ~8h faster than conventional testing
  - ID in 11h, AST in 9.6h
  - No MICs, just S/I/R
- Significant improvement in mortality, ICU LOS, ventilator days, # procedures, and costs, but not overall LOS.

Even rapid gram stain has a mortality impact

- Positive blood cultures

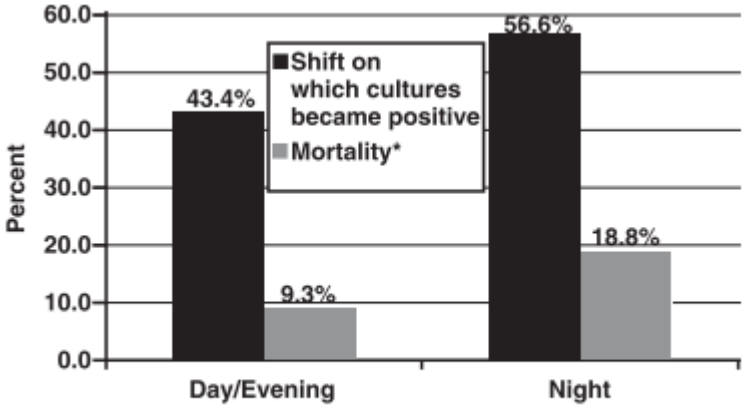


Figure 2 Culture positivity and mortality. * $P = .0624$.

	<1h TAT	≥1h TAT	Difference	P value
Time to detection (h)	13.7	13.6	0.1	0.7860
Gram stain TAT (h)	0.1	3.3	–3.2	<.0001
Mortality rate (%)	10.1	19.2	–9.1	0.0389
Length of stay (d)	11.0	10.5	0.5	0.6936
Positive length of stay (d)*	7.9	7.7	0.2	0.7920
Variable costs ($)	9,543	9,361	182	0.9150
Male sex (% of group)	47	49	–2	0.7773
Age (y)	69.2	66.6	2.6	0.3054

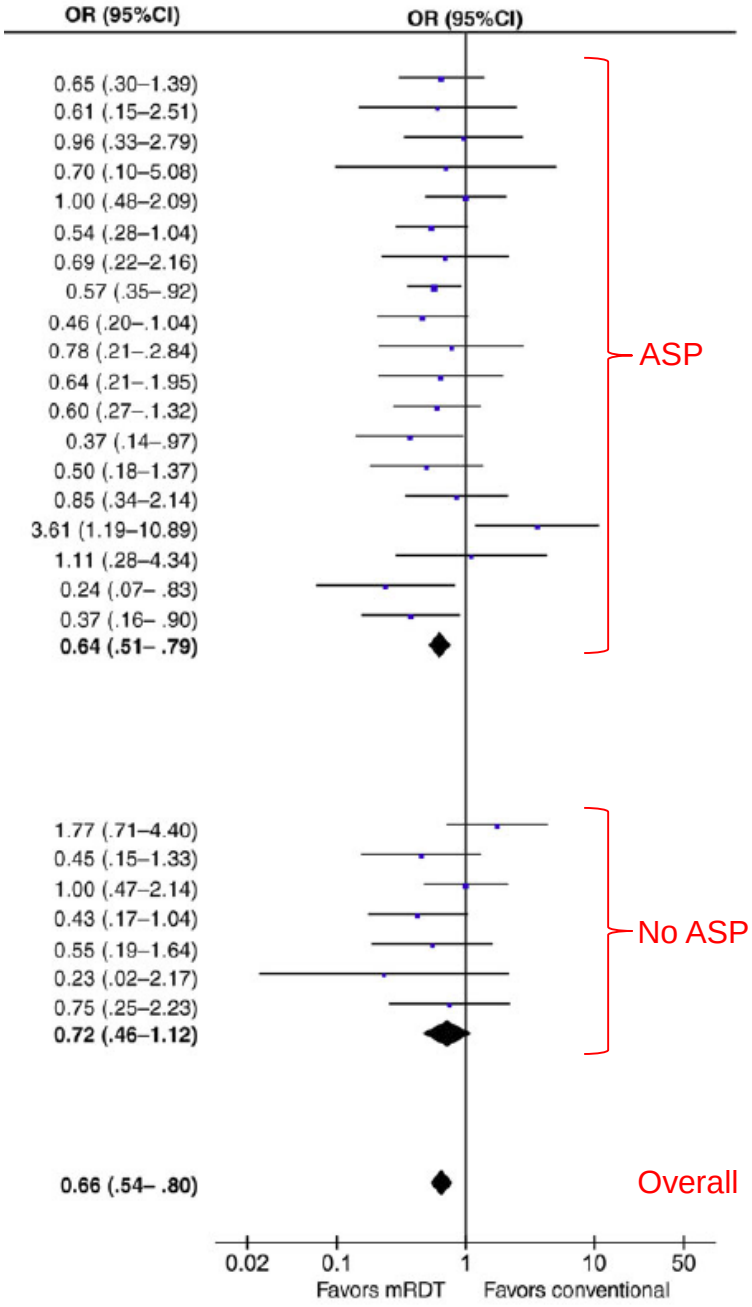
* The number of days between the date the culture became positive and the date of discharge.

- No difference in time to appropriate abx

Barenfanger08AJCP 130:870

Rapid molecular Dx?

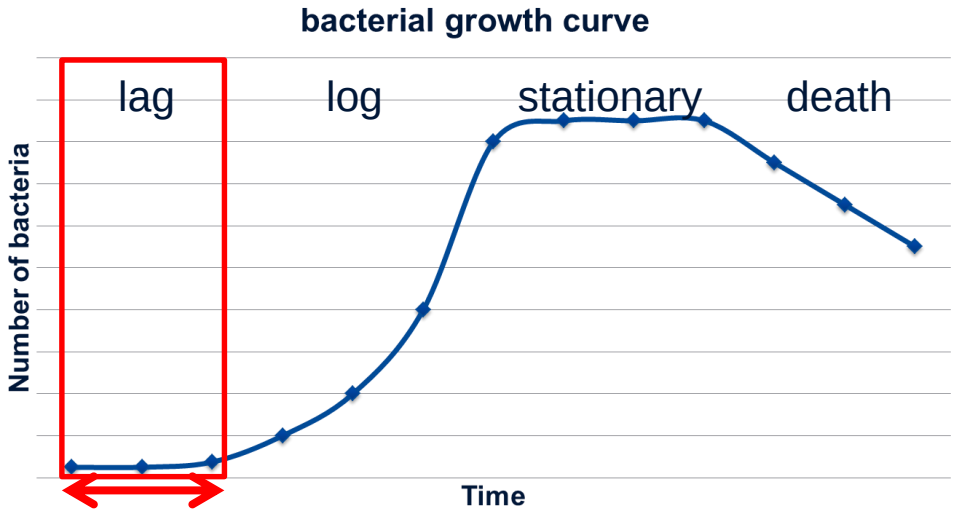
- Meta-analysis of mortality benefit in BSI, 31 studies, ~6k patients
  - Only 2 RCT, 2 case-control
- PCR, multiplex-PCR, MALDI-TOF, PNA-FISH from positive BC
- Numerical reduction of mortality with rapid identification (± "AST")
- Not statistically significant without accompanying antibiotic stewardship
  - *"To affect outcomes significantly, however, efficient clinical follow-up must be ensured…" Bruins05EJCMID*
- Overall, rapid results *do* have clinical impact
  - Time to results, and to a lesser extent, time to appropriate antibiotics are *typically* significantly better with rapid testing
  - Length of stay, costs are *often* significantly reduced
  - Mortality is frequently *not* significantly reduced
- **Can't expect a rapid molecular result alone to reduce mortality**



Timbrook17CID 64:15

Will rapid phenotypic AST be different?

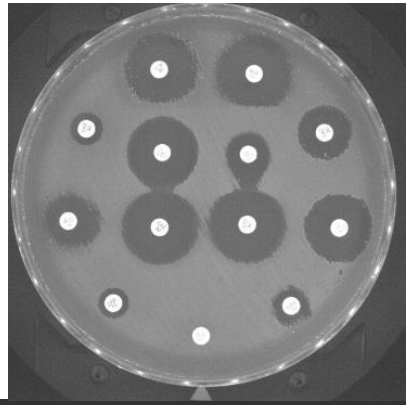
- How fast can it be?
  - Limited by growth rate
  - Curve is dependent on
    - Organism
    - Growth medium
    - Environment
  - Should be <4h (current commercial minimum)



- Will clinicians be more comfortable with these results than current partial/supplemental molecular tests?
  - Ideally ‘full panel’ results generated that do not need confirmation with traditional AST

Rapid Disk Diffusion

- Multiple studies since the 1970s
  - Reasonably high agreement at 4-8h vs. o/n reads, even directly from blood cultures
  - So why aren't we doing this every day? → Not "Standardized"?
- CLSI
  - Chandrasekaran et al: preliminary study
    - 20 GNR isolates, multiple labs, direct BC inoculum, read with current breakpoints at 6 and 18h
      - No dilution, washing, centrifugation, etc – just BC broth smeared on plate!
    - 20 drugs evaluated
    - CA was modest at 6h (~70%) vs. BMD, 20% were not readable at 6h
    - Studies ongoing to establish recommendations
- EUCAST Rapid AST (RAST)
  - Current guidelines for short incubation (4, 6, 8h) AST directly from BC bottles
  - Validated for the following species:
    - *Escherichia coli*
    - *Klebsiella pneumoniae*
    - *Pseudomonas aeruginosa*
    - Acinetobacter baumannii
    - *Staphylococcus aureus*
    - *Enterococcus faecalis* and *Enterococcus faecium*
    - *Streptococcus pneumoniae*
  - Limited # of drugs



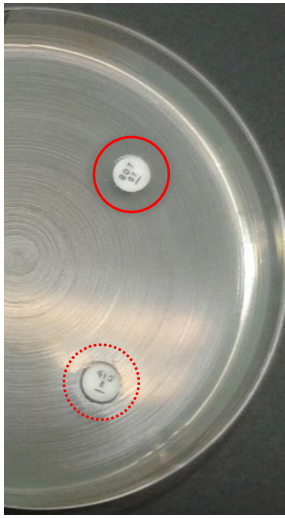
Chandrasekaran18JCM 56:e01678, Jonasson18ECCMID #O0747, http://www.eucast.org/rapid_ast_in_blood_cultures/

EUCAST RAST

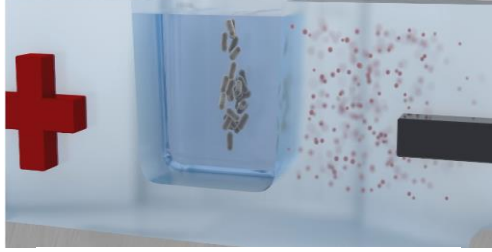
- Disk diffusion with early reads direct from positive BCs
  - Inoculate plates w/ pos BC fluid
  - Incubate on MH/MH-F agar
    - % readable at early timepoints
    - If zones not obvious, reincubate
    - Maximum incubation = 8h
  - Organism- and time-specific breakpoints
    - 4-8 drugs validated for each organism, more to come for GNRs
    - Need to know ID before reporting → Rapid molecular/MALDI-TOF
  - Area of Technical Uncertainty: less separation of S & R with short incubation. Report as "Susceptible, increased exposure"
  - During implementation, QC should be performed for the entire process: spike BC bottles containing sheep/horse blood, set up per protocol when flagged positive, evaluate using RAST-specific QC ranges

Organism	4h (%)	6h (%)	8h (%)
Escherichia coli	90	99	99
Klebsiella pneumoniae	96	98	98
Pseudomonas aeruginosa	-	88	97
Acinetobacter baumannii	99	100	100
Staphylococcus aureus	55*	91	95
Enterococcus faecalis	93	99	100
Enterococcus faecium	44	93	99
Streptococcus pneumoniae	68	83	95

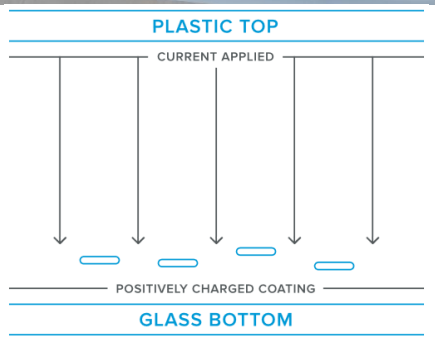
* Fox/gent easy, clinda/norflox harder

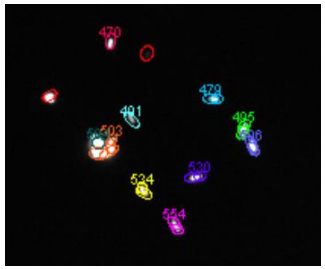


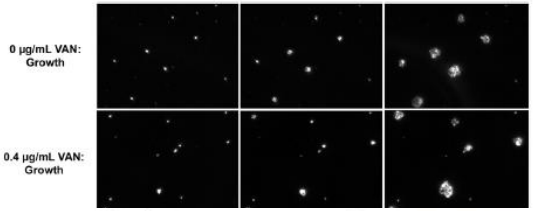
Accelerate Pheno BC



- FDA cleared system for automated ID/AST from positive blood cultures
- Gel electrofiltration cleanup and electrostatic immobilization of bacteria
- Automated quantitation and dilution
- Automated microscopy of cells grown with and without antibiotics
- ID in ~90 min (automated FISH, 6 G+, 8 G-, 2 yeast)
- AST in ~7h (8 G+, 12 G- drugs)
  - MIC extrapolated from growth characteristics
- 1 sample per instrument ($250/sample, $120k instrument list price)







***Klebsiella* spp.**
K. oxytoca
K. pneumoniae

***Enterobacter* spp.**
E. cloacae
E. aerogenes

***Proteus* spp.**
P. mirabilis
P. vulgaris

***Citrobacter* spp.**
C. freundii
C. koseri

CNS spp.
S. capitis
S. epidermidis
S. haemolyticus
S. hominis
S. lugdunensis
S. warneri

***Streptococcus* spp.**
S. agalactiae
S. gallolyticus
S. mitis
S. oralis
S. pneumoniae

E. faecium
Enterococcus spp. other than *E. faecalis*

Price14JMM 98:50, acceleratediagnostics.com

Accelerate performance

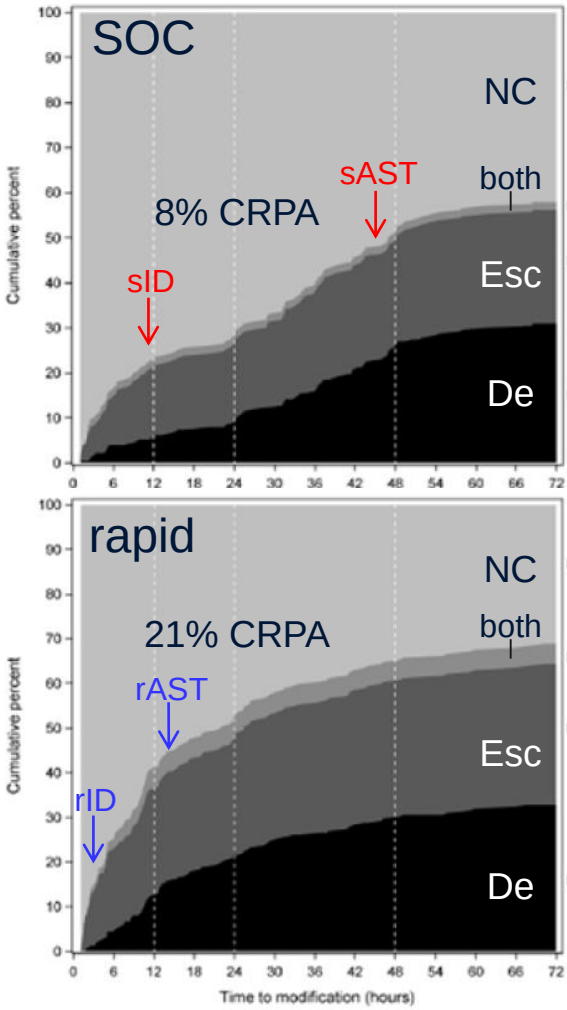
- Numerous analytical performance studies
  - Early problems with invalid results
    - software updates improved performance
  - Good categorical and MIC agreement
  - Faster than ‘standard of care’ AST
    - Most did not compare to ‘rapid’ standard AST (short incubation ‘scum’ plates, BC broth processing, direct disk diffusion)
- Outcome studies
  - Most have focused on ‘stewardship’ outcomes
  - Most showed reduced time to *optimal* therapy
    - Not always improvements in time to *active* therapy
      - ~70-90% of patients are on appropriate empiric Rx before testing
  - Some showed decreased time to Abx de-escalation/escalation

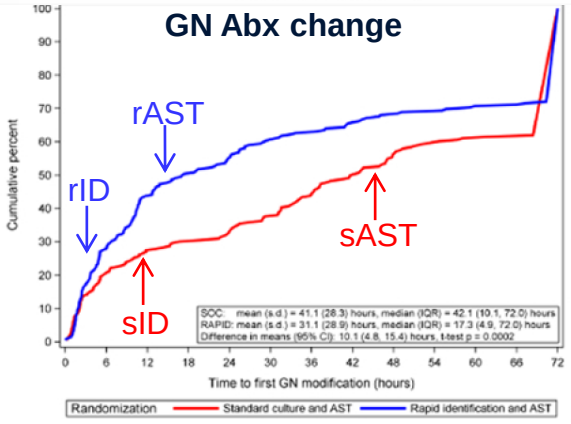
Brazelton deCárdenas17DMID 89:52, Marschal17JCM 55:2116, Charnot-Katsikas18JCM 56:e01166, Pancholi18JCM 56:e01329, Pantel18JAC 73:1546

Accelerate outcomes

- Pearson et al poster:
  - Pre-post intervention; 24-7 Accelerate testing (± Real-Time calls to ASP) vs. standard O/N subculture-based ID/AST.
    - Significant 'stewardship' outcomes: time to/# on optimal Rx (-1d), days of Rx (-0.8,-1.6d), broad GN Rx (-1.5d), broad GP Rx/Vm (-1d, RT-only), narrow β-lactam Rx (+1d, RT-only)
    - Overall LOS after BC collection decreased significantly (-0.6,-1.4d), but ICU LOS did not (+0.5,+0.6d)
    - Cost not evaluated: 19% off-panel → 17% polymicrobial (excl.) = ~1/3 of runs excluded. 46% CoNS.

- Banerjee et al poster:
  - Multi-center prospective RCT, Gram negative BSI
    - Sig lower time to 1st GN Abx mod/de/escalation
    - ICU duration, *C. diff*/MDRO aquisition, LOS, mortality: Not Significantly different
      - Rapid group: more in ICU at randomization, ↑ CRPA, ↑ LOS and ↑ mortality (NonSig).
      - Sicker patients in rapid group? Charleston comorbidity/Pitt bacteremia scores ~same





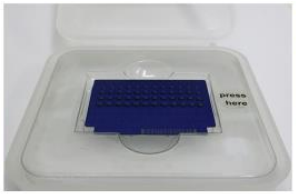
Pearson19IDWeek #2137; Banerjee19IDWeek#640

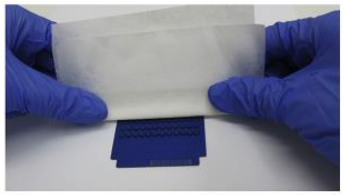
MALDI-TOF on-target AST

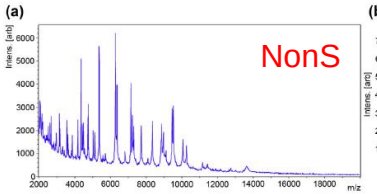
- Bruker MALDI Biotyper system
- Idelevich et al: Direct-On-Target Microbial Growth Assay (DOT-MGA)
  - **Proof of principle 1 (CMI-isolates):** *K. pneumoniae* and *P. aeruginosa* (24 ea) vs. 2µg/mL meropenem
    - 0.5 McF, dilute, mix w/ broth + mero, incubate on-target 3-18h
    - Liquid wicked off, dry, add matrix + protein std
    - Analyze with **standard ID software**: >1.7 ID score = growth (non-susceptibile)
    - 6 µL, 4h for *K. pne*, 5h for *P. aer*: 88-100% valid and 100% matched BMD (S vs. NonS)
  - **Proof of principle 2 (JCM-blood cx):** 28 enterics from spiked BC bottles vs. 2µg/mL meropenem
    - Compared 4 BC prep methods: dilution, filter-dilution, differential centrifugation, lysis-centrifugation
    - 1:10k dilution of BC, lysis-cent and diff cent had best composite performance
    - Dedicated software improved performance of lysis-cent to 96% valid, 100% sens/spec

Processing	Validity	Sens	Spec
10k dilution	92.6	90.9	100
Lysis-cent	96.3	91.7	100
Diff-cent	96.3	83.3	100

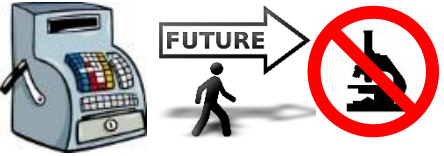
- Correa-Martinez et al: DOT-MGA for MICs!
  - **Proof of principle:** 50 enterics vs. ESBL/AmpC screening panel
    - Growth patterns ± ESBL/AmpC inhibitors predicts resistance mechanism (EUCAST)
    - 94-100% pos/neg agreement with PCR after 4h; better than BMD or disk testing at 18h
- Bonus: like rapid DD, you may already have this capability in your lab!





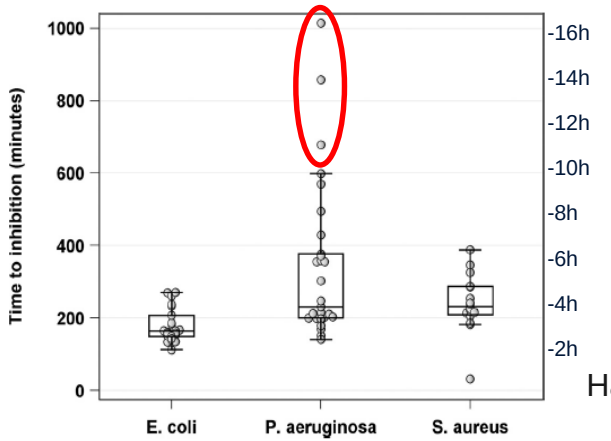


Idelevich18CMI 24:738, Idelevich18JCM 56:e00913, Correa-Martinez19FrontMicro 10:13

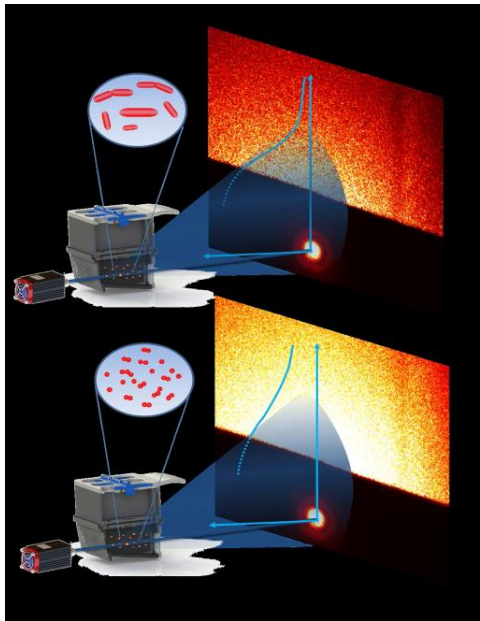


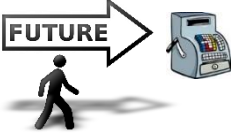
Bacterioscan

- BacterioScan 216Dx UTI System
  - Optical density + forward laser scatter
    - Information on culture density and size/shape of bacterial cells
    - Accurate quantification
  - FDA-cleared instrument for pos/neg UTI calls – **no AST yet**; $20/cuvette, $25k instrument
  - 16 tests/instrument = breakpoint panels or few drugs
- BacterioScan 216R Rapid AST System in development
  - Hayden et al:
    - Proof-of-principle, 3 isolates each: *E. coli, P. aeruginosa, S. aureus.*
    - 72/89% agreement with Vitek2/Microscan
    - 80% bug/drug combos interpretable <6h
  - Idelevich et al:
    - MRSA/MSSA and VRE/VSE, 50 isolates each
    - 98-100% sens, 92-94% spec; real-time curve data



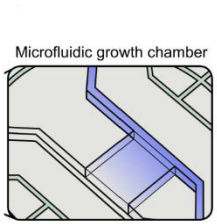
Hayden16JCM 54:2701, Idelvich17FrontMicro 8:1064
bacterioscan.com

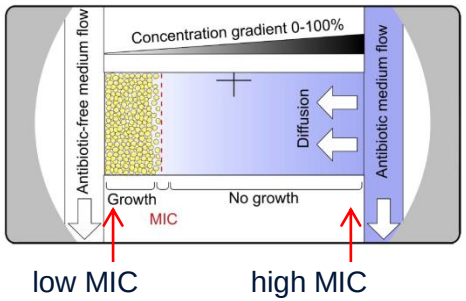


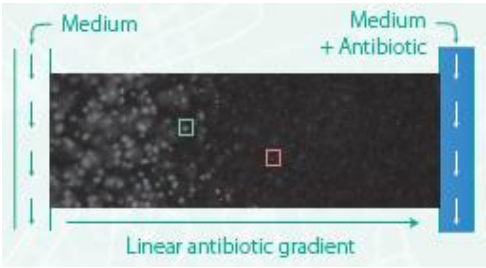


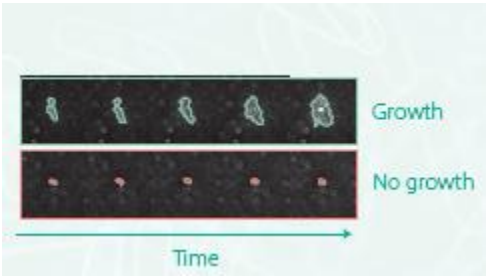
Gradientech

- Time-lapse microscopy, microfluidics
  - Suspend culture in agarose, auto-load into analysis cells (12 drugs + ctrl), auto-image analysis
- MICs derived from linear drug gradient
  - Change in greyscale (microcolonies) across cell
  - Analogous to Etest
- 2-5h AST from positive blood cultures
  - 1 specimen/module, ~$35/test, ~$13k/module
  - Unstandardized inoculum (spin→supe), can do isolates
  - Initially planned CE 2019, FDA 2020
- Malmberg et al:
  - Prototype/proof of principle; QC orgs and 13 +BC compared to Etest and broth macrodilution
  - 100% EA at 10^5 cfu/ml, 77% from blood cultures
    - BC lower due to variable concentrations?







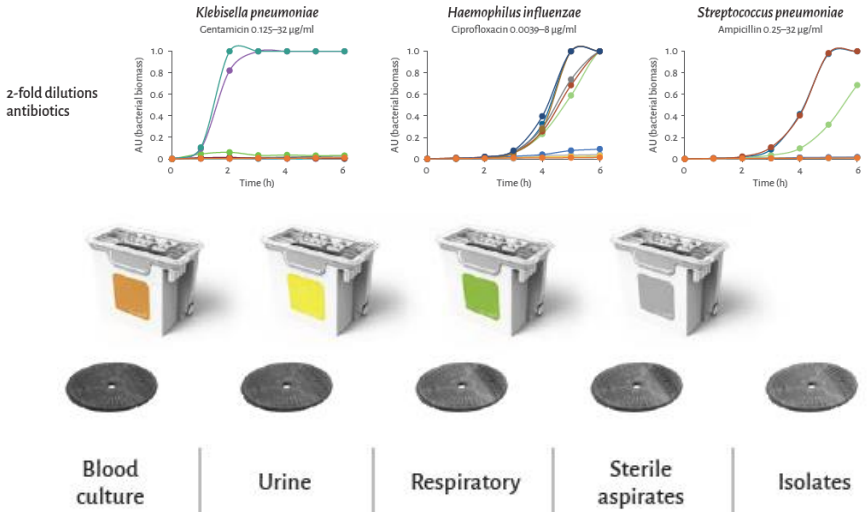


Malmberg16PLoSOne 11:e0167356, gradientech.se

Q-Linea ASTar

- Time-lapse microscopy, automated sample processing
  - Fully-automated processing, analysis
    - Direct from positive blood cultures and isolates, other specimens planned
    - ~1min hands-on time
  - 3 to 6 hours, true MIC
  - 6-12 samples/instrument, random-access
    - Up to 50 samples per day
  - Up to 48 drugs, 5-11 two-fold dilutions
  - Can test fastidious species
  - Clinical trials begin 2nd half of 2020; version with ID + AST in development

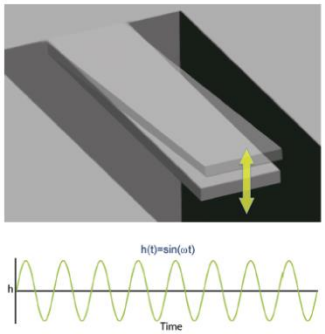
- Klintstedt et al poster
  - Prototype/proof of principle; genuine (26) and spiked (~85) +BC
  - 92-96% EA, 93-97% CA; ceftaz 83% EA/CA, ceftolozane-tazo 75% EA

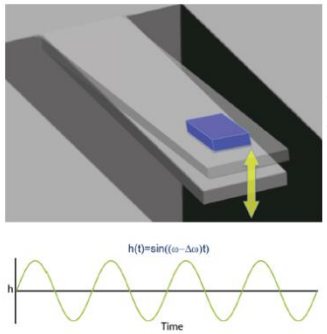


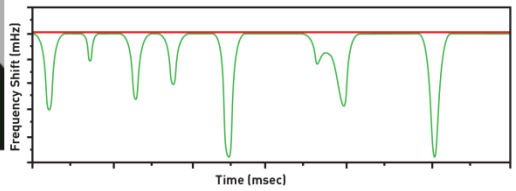
Klintstedt18Microbe #218, #206, qlinea.com

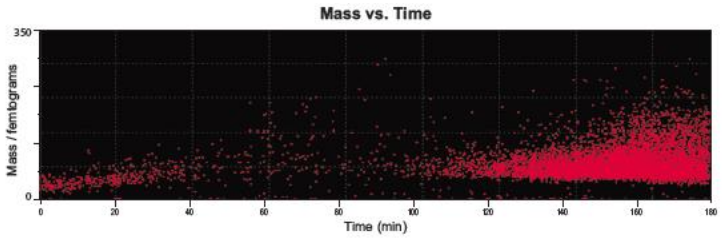
Lifescale

- Resonant mass measurement + cell counting
  - Bacterial cells reduce vibration frequency
  - Mass resolution ~1fg (~1% bacterial cell mass)
- Standard broth microdilution format (true MIC), 1 sample/instrument*
  - 100s-1000s of cells measured/well, ~35-60min read time/plate
  - ~$125/test; $125k/instrument
- 2-3.5h avg most GNR (some, incl. *P. aeruginosa*, may take longer)
- Schneider et al poster
  - Proof-of-principle, 58 GNRs – QC and test isolates; Sensititre MIC panels, reference BMD
  - 95% within QC range (on-panel), 19/24 drugs ≥90% EA, 22/24 drugs ≥90% CA; ceftaz, ceftriax EA/CA 81-88%









lifescaleintruments.com, Schneider16ASM-Microbe 024, *off-line incubation possible

Lifescale

- Positive blood culture panel
  - Gram negative rods
  - “simple centrifugation” sample prep
  - 14 antibiotics, MIC format
  - Interpretation based on external ID
  - On-scale QC
  - CE-marked
  - Clinical trials ongoing

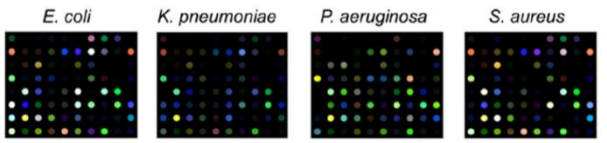
	1	2	3	4	5	6	7	8	9	10	11	12
A	MERO 0.125	FEP 0.5	AMI 4	ETP 0.125	LEVO 0.25	P/T 4	FAZ 0.25	AZT 1	TAZ 1	AMP 2	GEN 1	VAB 0.5
B	MERO 0.25	FEP 1	AMI 8	ETP 0.25	LEVO 0.5	P/T 8	FAZ 0.5	AZT 2	TAZ 2	AMP 4	GEN 2	VAB 1
C	MERO 0.5	FEP 2	AMI 16	ETP 0.5	LEVO 1	P/T 16	FAZ 1	AZT 4	TAZ 4	AMP 8	GEN 4	VAB 2
D	MERO 1	FEP 4	AMI 32	ETP 1	LEVO 2	P/T 32	FAZ 2	AZT 8	TAZ 8	AMP 16	GEN 8	VAB 4
E	MERO 2	FEP 8	AMI 64	ETP 2	LEVO 4	P/T 64	FAZ 4	AZT 16	TAZ 16	AMP 32	GEN 16	VAB 8
F	MERO 4	FEP 16	AMI 128	ETP 4	LEVO 8	P/T 128	FAZ 8	AZT 32	TAZ 32	AMP 64	GEN 32	VAB 16
G	MERO 8	FEP 32	AMI 256	ETP 8	LEVO 16	P/T 256	FAZ 16	AZT 64	TAZ 64	CZA 8	CZA 16	CZA 32
H	MERO 16	FEP 64	SXT 0.25	SXT 0.5	SXT 1	SXT 2	SXT 4	SXT 8	CZA 2	CZA 4	POS 0	POS 0

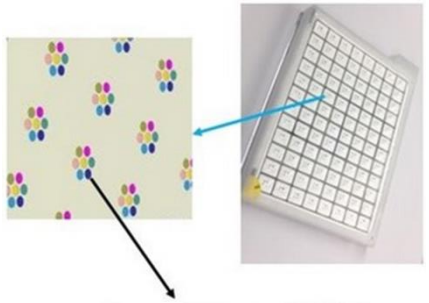
Growth Determination

Antibiotic (Panels)	Antibiotic Concentration	LS Concentration	Growth (Relative)	Grow/No Grow: Grow	Grow/No Grow: No Grow
Amikacin	8	9.05e+05	1.463879419		
	16	5.27e+05	0.852498249		
	32	3.51e+05	0.568136633		
	64	4.21e+05	0.680526035		
Ampicillin	4	2.23e+07	36.108113016		
	8	2.87e+07	46.477172006		
	16	3.00e+07	48.529097744		
	32	2.76e+07	44.711051278		
Ampicillin/Sulbactam	4	2.57e+07	41.632923583		
	8	6.59e+06	10.658191840		
	16	7.79e+05	1.260586576		
	32	3.84e+05	0.621812265		
Aztreonam	2	5.75e+05	0.929495336		
	4	1.25e+06	2.028572997		
	8	1.56e+06	2.521264693		
	16	1.48e+06	2.396693694		
	32	1.36e+06	2.203947495		
Cefazolin	0.5	3.00e+07	48.529097744		

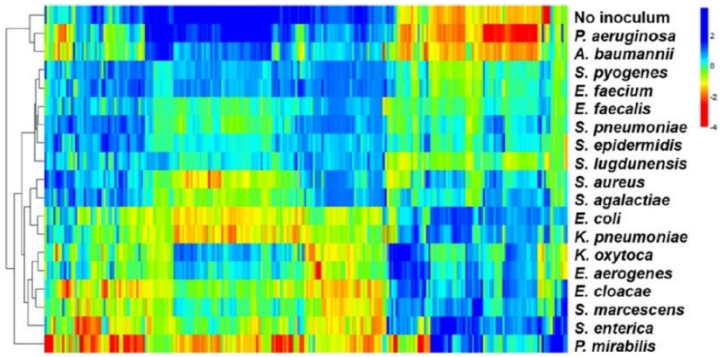
Specific Reveal-AST

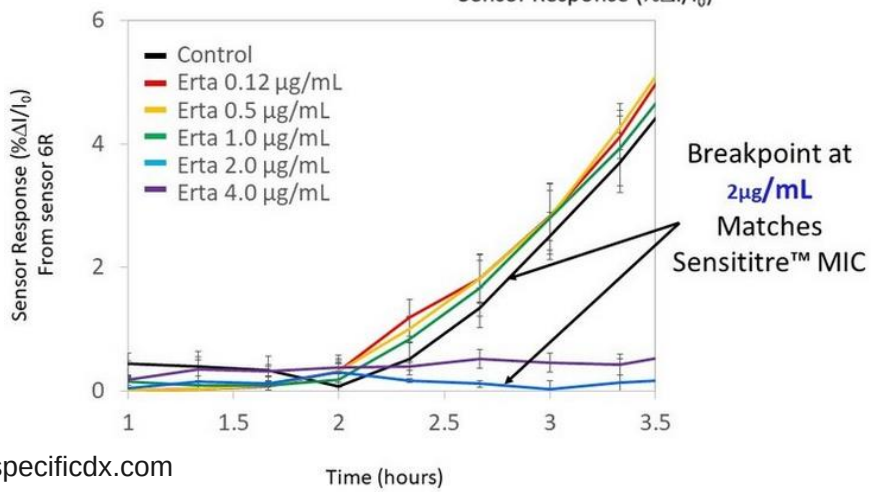
- Volatile Organic Compound detection
  - Colorimetric Sensor Arrays detect headspace VOCs over time
  - Direct from +BC (dilute→test) or isolates
  - 3-4h avg time to results, MIC format
  - Inexpensive – FIND/NIH funding for resource-limited setting platform





- Singh et al, poster
  - Proof of principle, 29 spiked BC bottles
  - 100% EA, 97% CA vs. BMD in ≤ 3h
  - ID *for free* by 4h from growth control

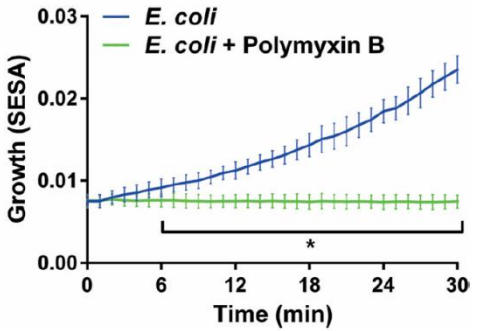


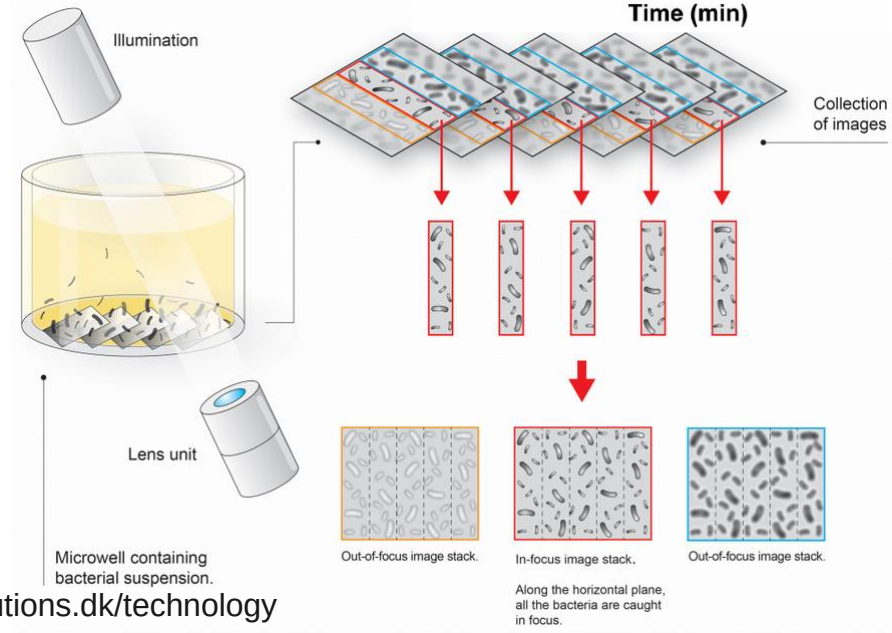


Lonsdale13PLoSOne 8:e62726, Lim14JCM 52:592, Singh17Microbe CPHM LB1, specificdx.com

oCelloScope

- Angled bright-field microscopy
  - 6.25° tilt improves performance over broader concentration range; volume, phase information
  - Z-stack of images, automated detection of in-focus region
  - 96-well MIC format, 1 sample/instrument
  - 1-4.5h avg time to results
  - No plans for IVD approval
- Fredborg et al 2015:
  - Proof-of-principle, 16 samples
    - QC, clinical isolates, and +BCs
    - 93% overall EA/CA
    - 95% of results in <3h (avg 100min)

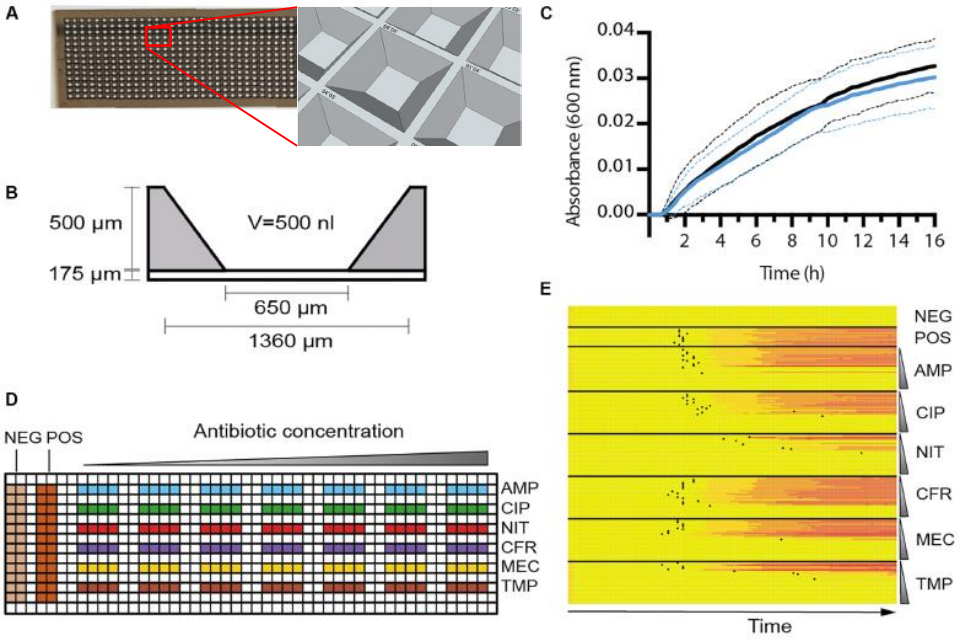




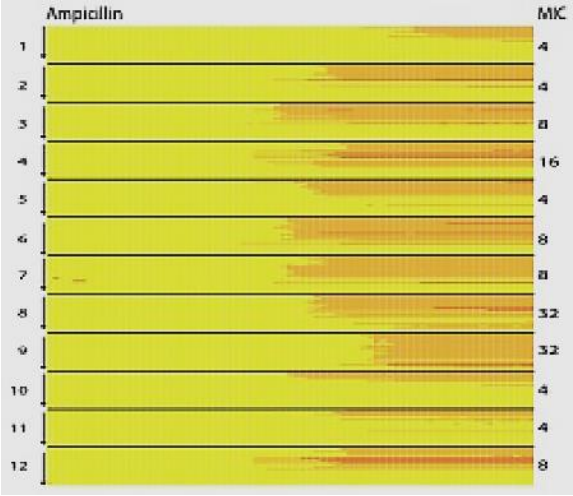
Fredborg13JCM 51:2047, Fredborg15EJCMID 34:2385, biosensesolutions.dk/technology

Nanowell AST

- nwAST (Broth nanodilution?)
  - Etched silica wells (672) attached to standard glass slide
  - Standard BMD conditions except 500nl wells, automated A_{600}
    - Compatible with imaging
  - 5-6h, true MIC, with replicates
    - time to growth drug vs. no drug (ΔT_{lag})



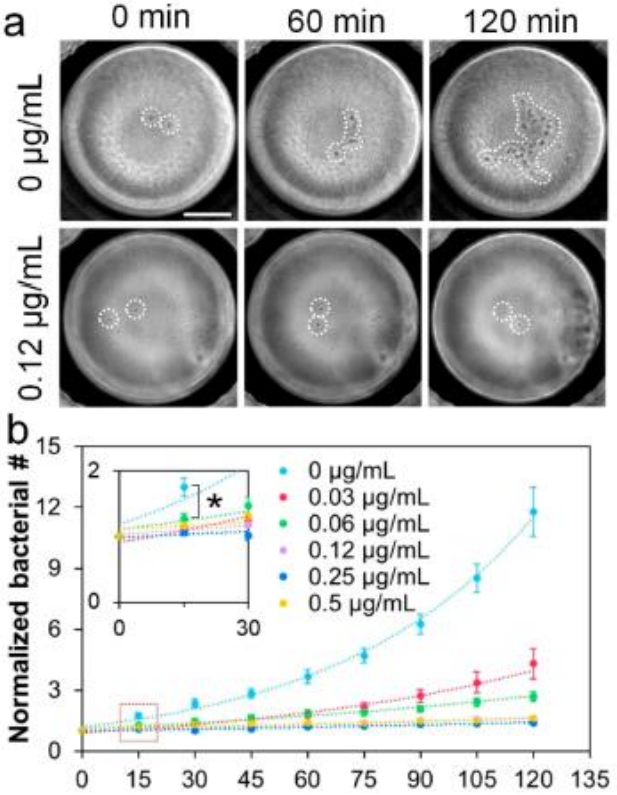
- Veses-Garcia et al
  - Prototype/proof-of-principle
  - 70 UPEC isolates, nwAST vs. disk diffusion
  - 98% overall CA; amp 8% false R
  - 5 other UTI pathogens grow well in nanowell format
  - More variable than desired

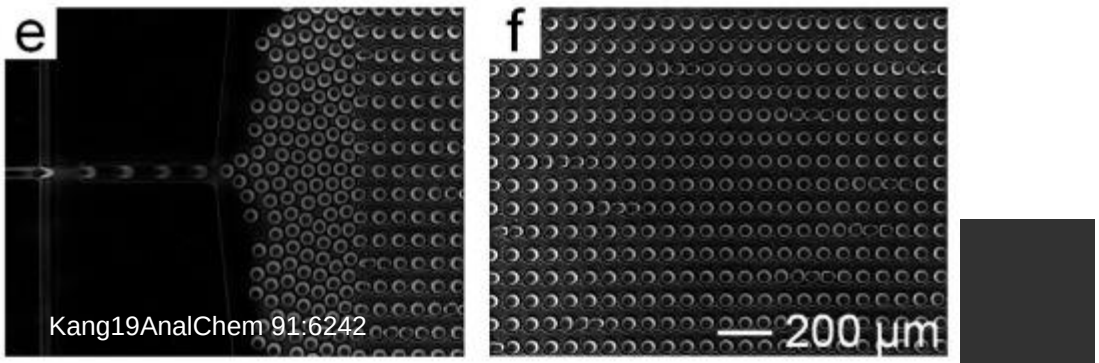


Weibull14JCM 52:3310, Veses-Garcia18FrontMicro 9:1530

Nanodroplet AST

- Kang et al
  - Prototype/proof of principle
  - 8000 ≤60μm droplets x 4 separate cells
  - 4 drug concentrations per unit
  - Very rapid (<60 min)
  - Individual droplet and cell analysis
    - Time lapse microscopy ≥ 100 per condition
    - Statistics
  - Limited testing to date
    - *S. aureus*, *E. faecalis* vs. oxacillin
    - *E. coli, K. pneumoniae* vs. tetracycline





Kang19AnalChem 91:6242

Summary

- Current rapid molecular “AST” has a measurable, but not always significant effect on patient outcomes
  - May not be substantial enough to overcome empiric choices
  - Faster probably won’t help
  - More information *may* help
- Rapid phenotypic AST methods in development hope to fill this gap
  - Commercial systems with full, final results in <4h may be available soon
    - 4-12h already available: Vitek, Phoenix, Microscan. Set up from BC ‘scum’ plate.
    - Accelerate, ≤7h to fairly complete AST results
      - FDA cleared for positive blood cultures
  - Single-cell or micro/nano-scale methods can improve time to results
  - Direct from specimen is the ultimate goal
    - Not there yet, but direct from urine testing is likely
    - Imaging methods hold promise: analyze mixed morphotypes
- Regardless of method, work with stewardship and other stakeholders to maximize impact of rapid AST