The Current Landscape of Antimicrobial Resistance Testing in the United States

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Conflicts of Interest:

- None

Objectives:

- Be able to discuss the current processes in use for standard antimicrobial susceptibility testing.
- Various methods for antimicrobial resistance testing as well as recommendations for confirmation in the context of case studies.
- Current and future options for rapid antimicrobial susceptibility testing and how these processes could improve the time to a result.

Outline:

- Key concepts for a reliable antibiotic susceptibility testing result.
- AST process for isolated colonies.
- Antimicrobial resistance (AMR) cases.
- Brief overview of direct from specimen/rapid processes currently available or in development.

Objective of Antibiotic Susceptibility Testing (AST):

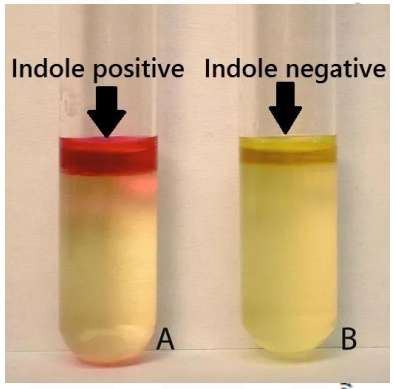
- To detect acquired antibiotic resistance of a particular isolate when compared to the wild-type isolate susceptibility profile.

- Intrinsic resistance is a natural resistance that is present in the wild-type isolate.
  - The antibiotic will not be clinically effective even if it appears susceptible *in vitro.*

Identification is essential to AST interpretation

If the organism has not be identified, then the ability to interpret biochemical mechanisms of resistance as well as intrinsic resistance becomes impossible. (Antibiogram. Cuorvalin, et al. ISBN: 978-1-555-81496-0,2010 ASM Press)

CLSI M35-A2: Abbreviated Identification of Bacteria & Yeast:



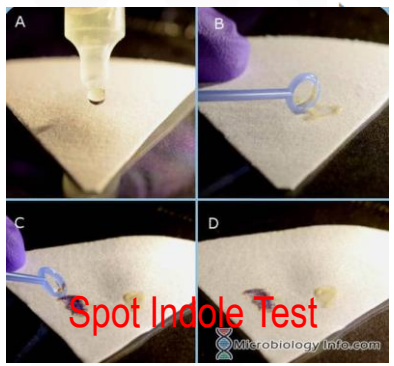
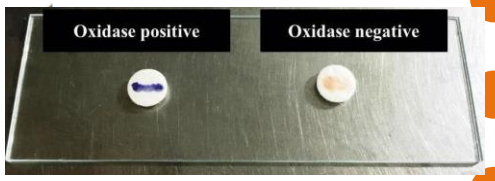
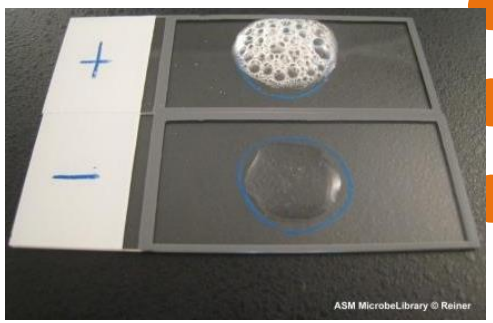
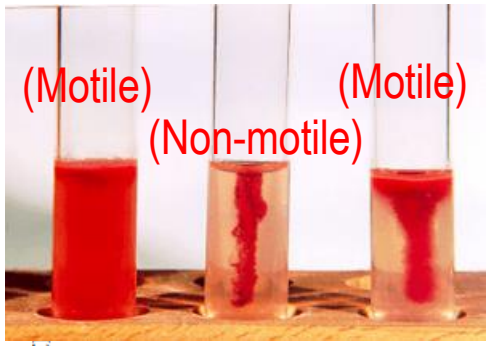


Table 1. Common Pathogens and Rapid Methods to Identify Gram-Negative Organisms When Suspected From Colony Morphology

Organism	Presumptive Identification	Additional Tests for Definitive Identification	Additional Notations
Brucella	1. Tiny gram-negative coccobacilli 2. Oxidase positive 3. Catalase positive 4. MacConkey negative	1. Urea positive 2. Indole negative 3. Nonhemolytic on BAP	Work in safety cabinet; highly infectious PDA negative Found in sterile tissues and fluids Forward to LRN reference laboratory.
Campylobacter jejuni/coli	1. Gram-negative rod with gull wings 2. Oxidase positive 3. Catalase positive 4. Darting motility	1. Hippurate positive (*C. jejuni*); or 2. Indoxyl acetate positive (*C. jejuni/coli*)	Must be isolated from stool growing at 42 °C on *Campylobacter* selective medium.
Cardiobacterium hominis	1. Pleomorphic thin gram-negative rod 2. Oxidase positive 3. Catalase negative 4. MacConkey negative	1. Indole positive 2. Nonhemolytic	Isolate must be from blood culture. Confirm with negative nitrate test.
Eikenella corrodens	1. Small gram-negative rods 2. Grows as colonies that pit agar on BAP or chocolate in CO_2 3. Oxidase positive 4. Catalase negative 5. MacConkey negative	1. Indole negative 2. Nonhemolytic 3. Distinct odor of bleach	Ornithine positive if atypical colonies.
Escherichia coli	1. Oxidase negative 2. Indole positive 3. Gram-negative rods by stain or growing on gram-negative selective agar	1. Hemolytic; or 2. Lactose positive and PYR negative; or 3. MUG positive	Isolate must be growing as large colonies and not from gastrointestinal site specimen.
Francisella tularensis	1. Tiny gram-negative rod or coccobacilli 2. Oxidase negative 3. Catalase negative or weak 4. Slow growth on chocolate, but no growth on BAP even around *Staphylococcus* streak	Beta-lactamase positive	Work in safety cabinet; highly infectious Forward to LRN reference laboratory.







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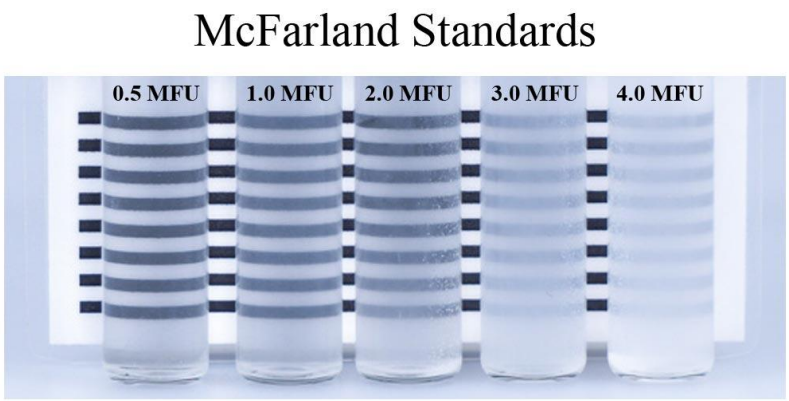
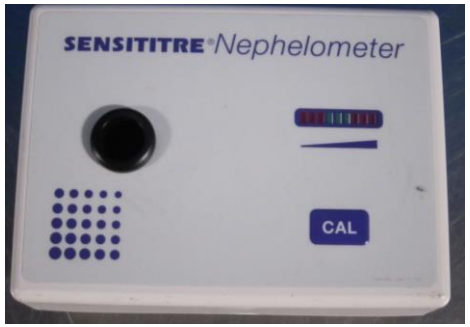
MIC.21940: Standardized Inoculum.

- The inoculum used for antimicrobial susceptibility testing (i.e., inoculum size) is controlled using a turbidity standard or other acceptable method.
  - NOTE: Antibiotic susceptibility may be substantially affected by inoculum size.
- 0.5 McFarland Standard = 1.5 x 10^8 CFU/mL





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Standardized Inoculum & Isolate Age

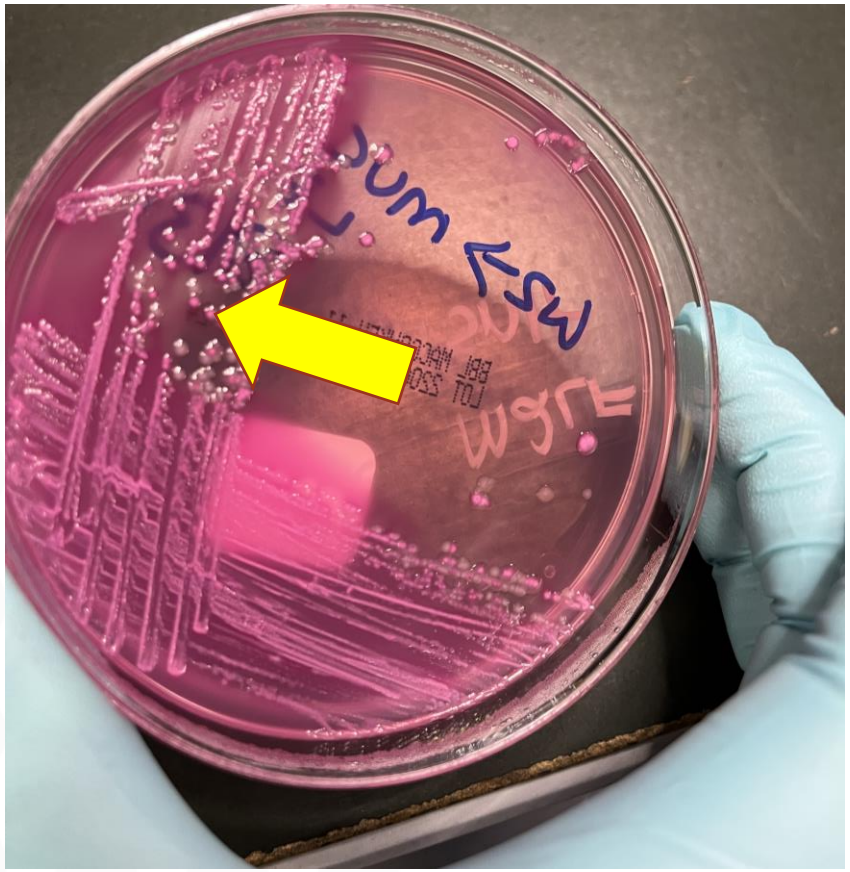
- 0.5 McFarland = utilized directly for setup of disk diffusion and ETEST/MTS strips.
- A dilution of the 0.5 McFarland to equal roughly 0.5 x 10^5 CFU/mL is utilized for macrobroth or microbroth dilutions.
- Troubleshooting opportunity: inoculum effect.
- Isolate age is recommended to be 18 to 24 hours. Comparability studies would be required to reduce this time.
- If utilizing a commercial system, follow manufacturer's instructions for inoculum density and age of the isolate.

Colony Selection:

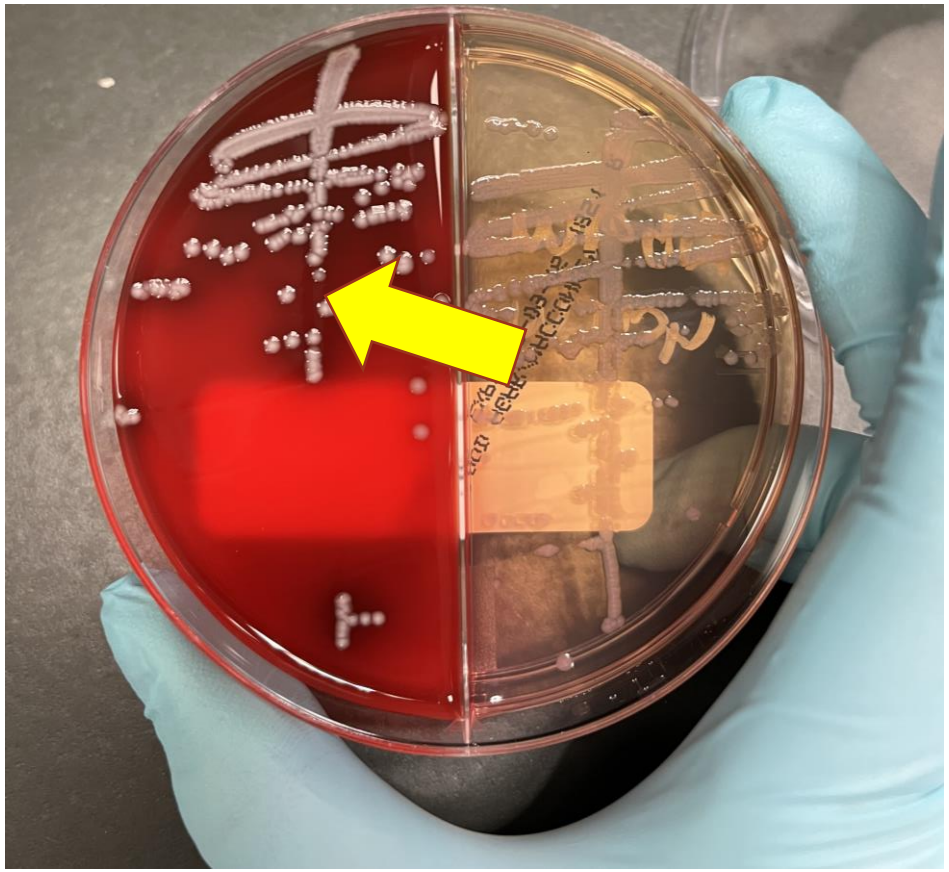
- CFU (Colony-Forming Unit): A colony represents one live bacterial cell that has multiplied until visible.
- Select well isolated colonies.
- MIC.21820: Susceptibility Testing – Pure Cultures
  - Antimicrobial susceptibility testing of isolates must be performed using pure isolates or colonies (i.e., susceptibility testing is not performed on mixed cultures).
  - NOTE: A purity check must be performed by subculturing an aliquot of the inoculum onto a blood agar plate or other non-selective media at the same time the inoculum is used for susceptibility testing with some exceptions.

Purity Plate:

- MAC: 2 different types of GNR



- BAP: 2 different types of GNR

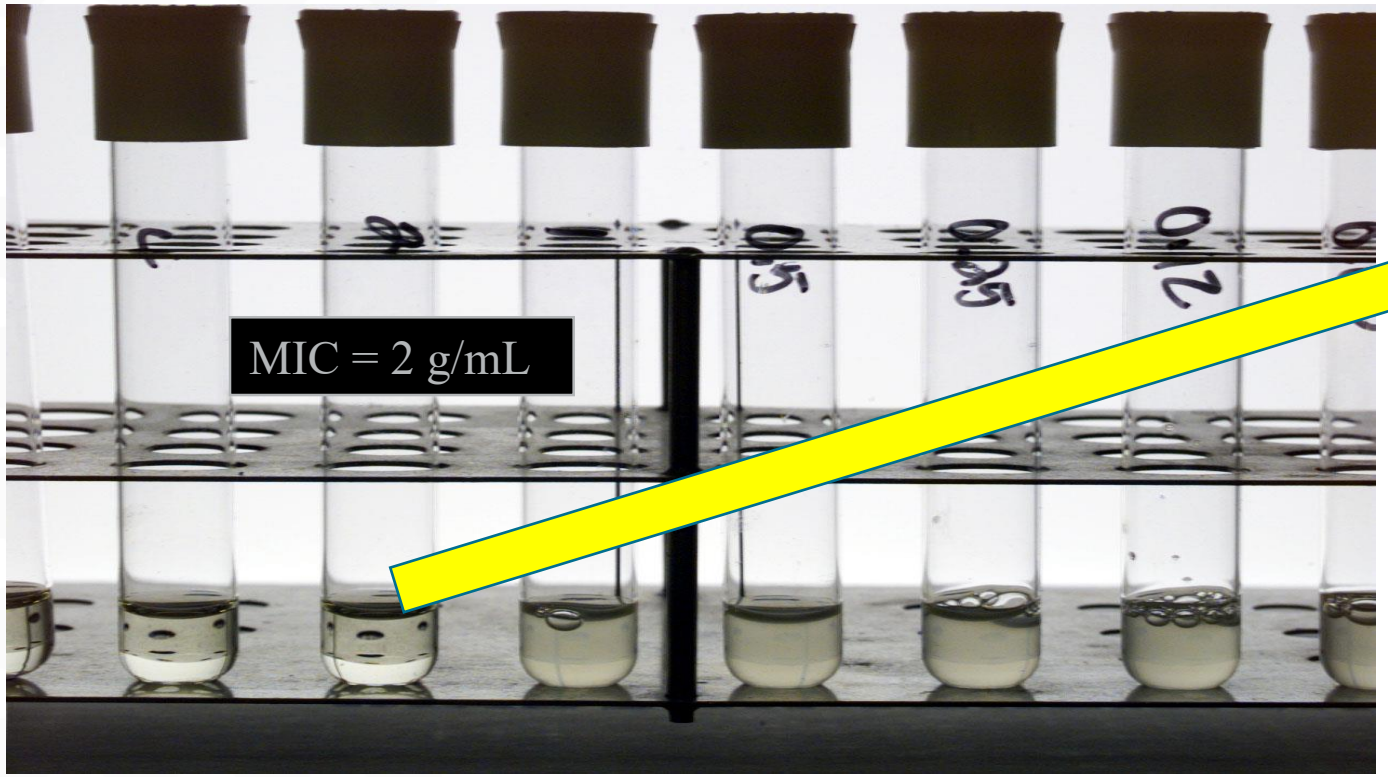


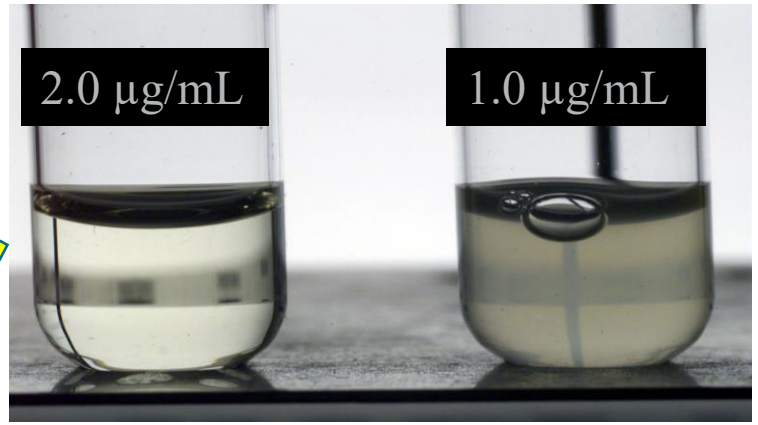
Antimicrobial Susceptibility Testing Processes

- AST for isolated colonies:
  - Macrobroth dilution
  - Microbroth dilution
  - Agar dilution
  - Disk diffusion (KB disk)
  - Gradient diffusion (ETEST/MTS): Epsilometer test
  - Automated/semi-automated

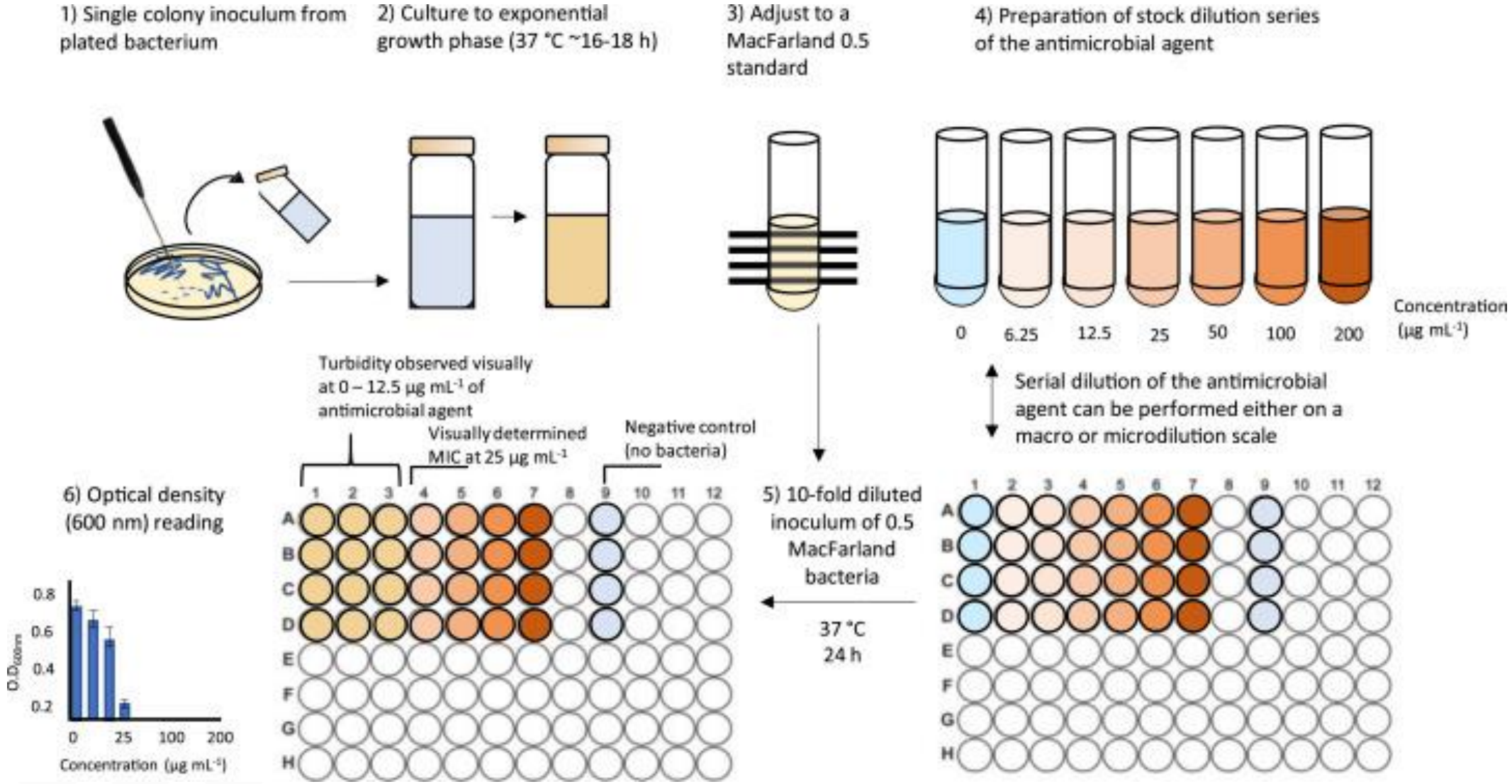
Minimum Inhibitory Concentration (MIC):

- Lowest Concentration of Antimicrobial that Visibly Inhibits Growth.
  - Example: Visible growth at 1 µg/mL; no visible growth at 2 µg/mL. MIC = 2 µg/mL.





Microbroth Dilution: Reference Standard



https://www.sciencedirect.com/topics/engineering/dilution-method

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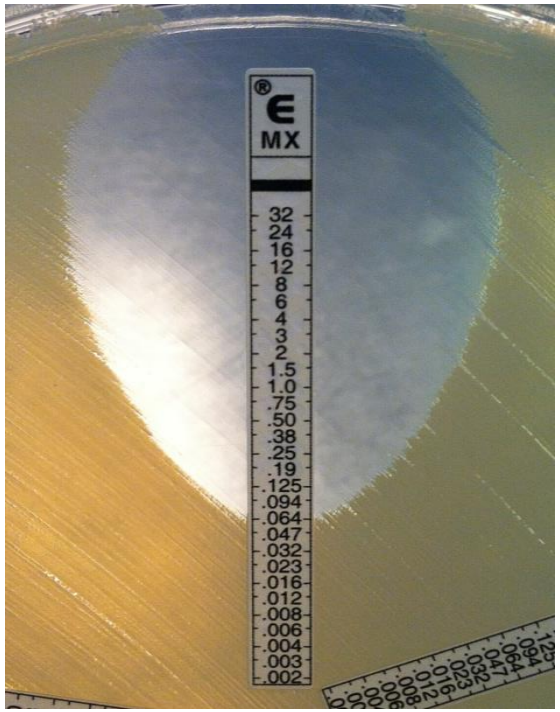
Gradient Strip: commercial ETEST/MTS

- Strip impregnated with a pre-defined concentration gradient of an antimicrobial agent, which is used to determine the minimum inhibitory concentration (MIC).
- 0.5 McFarland utilized. Expect a confluent or almost confluent lawn of growth.
- Reading: observe where the relevant inhibition ellipse intersects the strip and read the MIC at complete inhibition. Follow manufacturer's instructions in regards to reading specific antibiotic strips and if ≥ or <.
- Utilize susceptibility interpretative criteria as recommended by the FDA or standards development organization.



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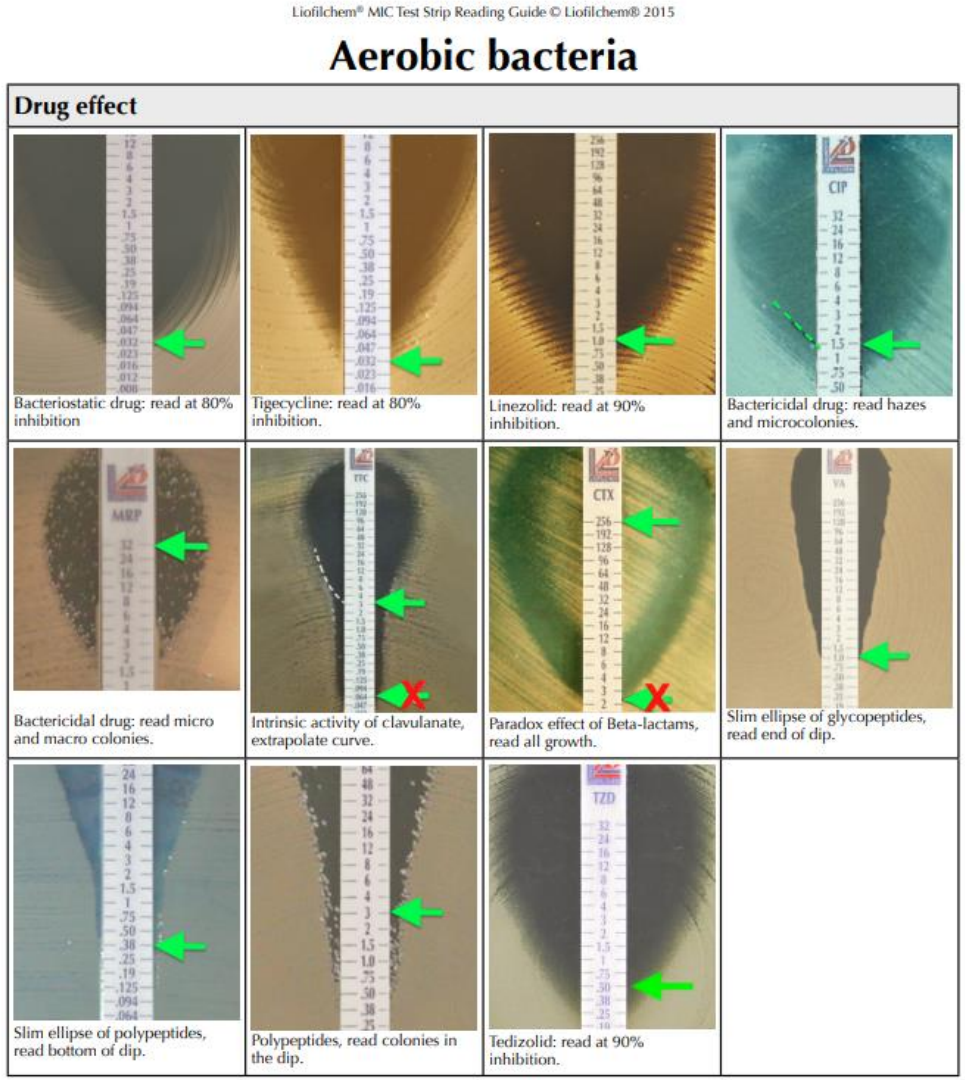
Gradient Strip: MIC interpretation instructions required



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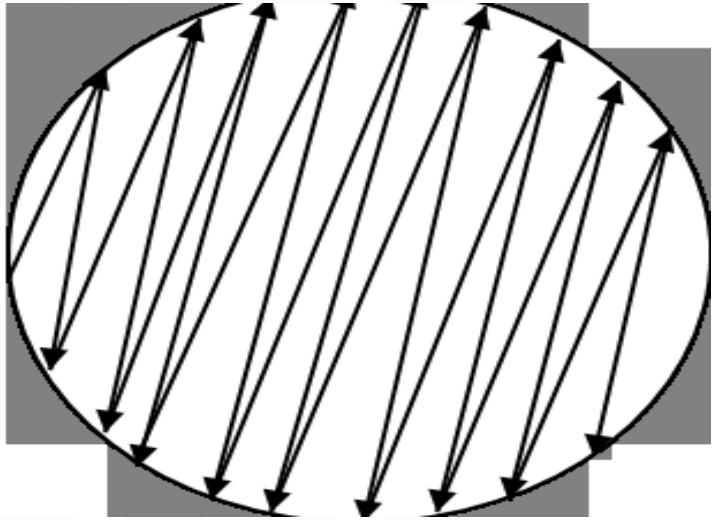
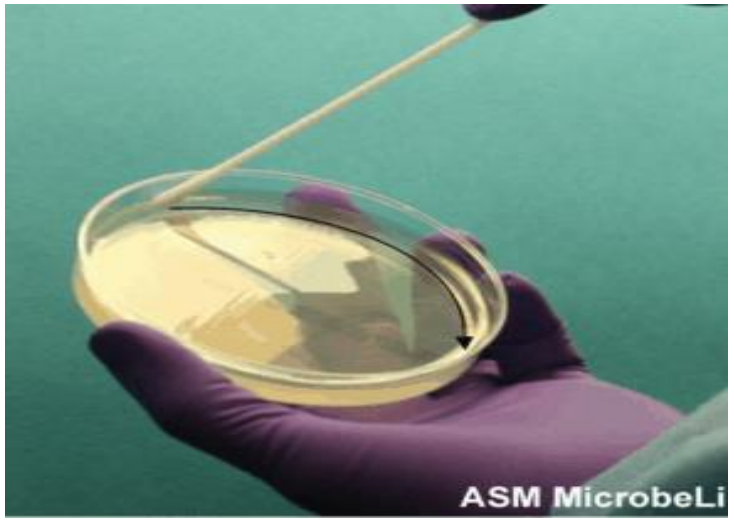
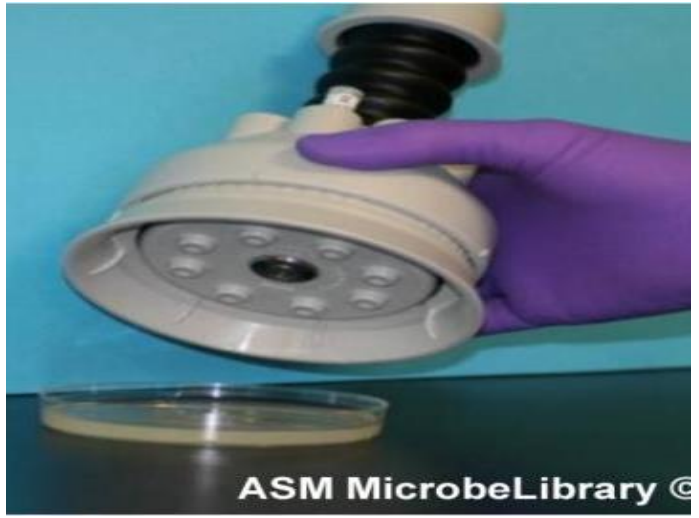
Disk Diffusion (Kirby-Bauer disk)

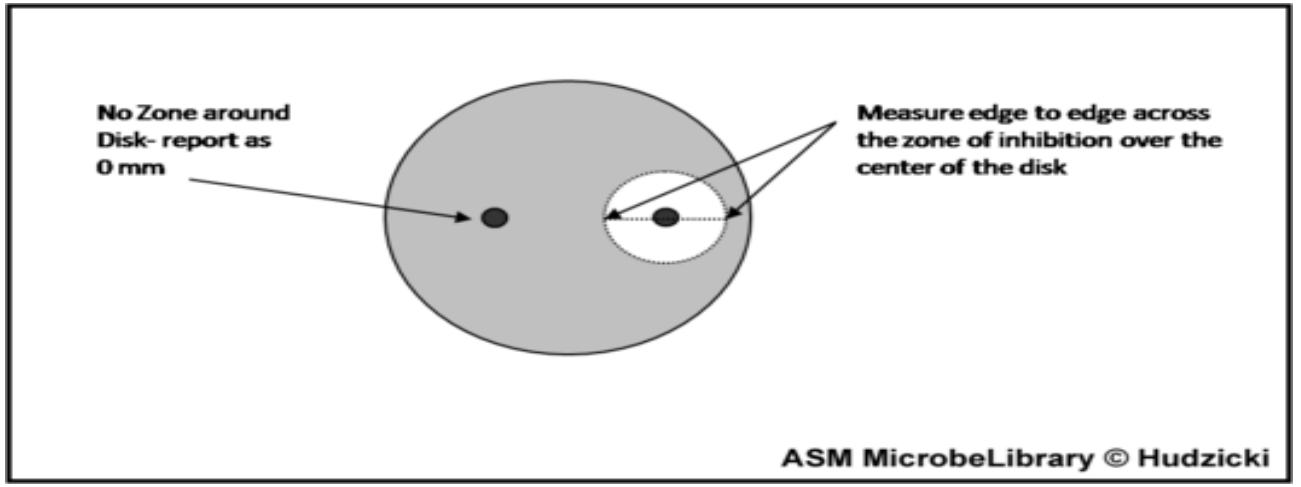
- Qualitative Method: RR, SS, I, SDD, NS
- Interpretation only option. If an interpretation is not established by FDA and/or standards development organization then this method is not valid.
- Excellent method for cost efficiency and for phenotypically noting new types of resistance patterns.
- Added benefit for laboratories with Total Laboratory Automation for measuring the DD as well as some modules being able to perform setup.

Disk Diffusion:









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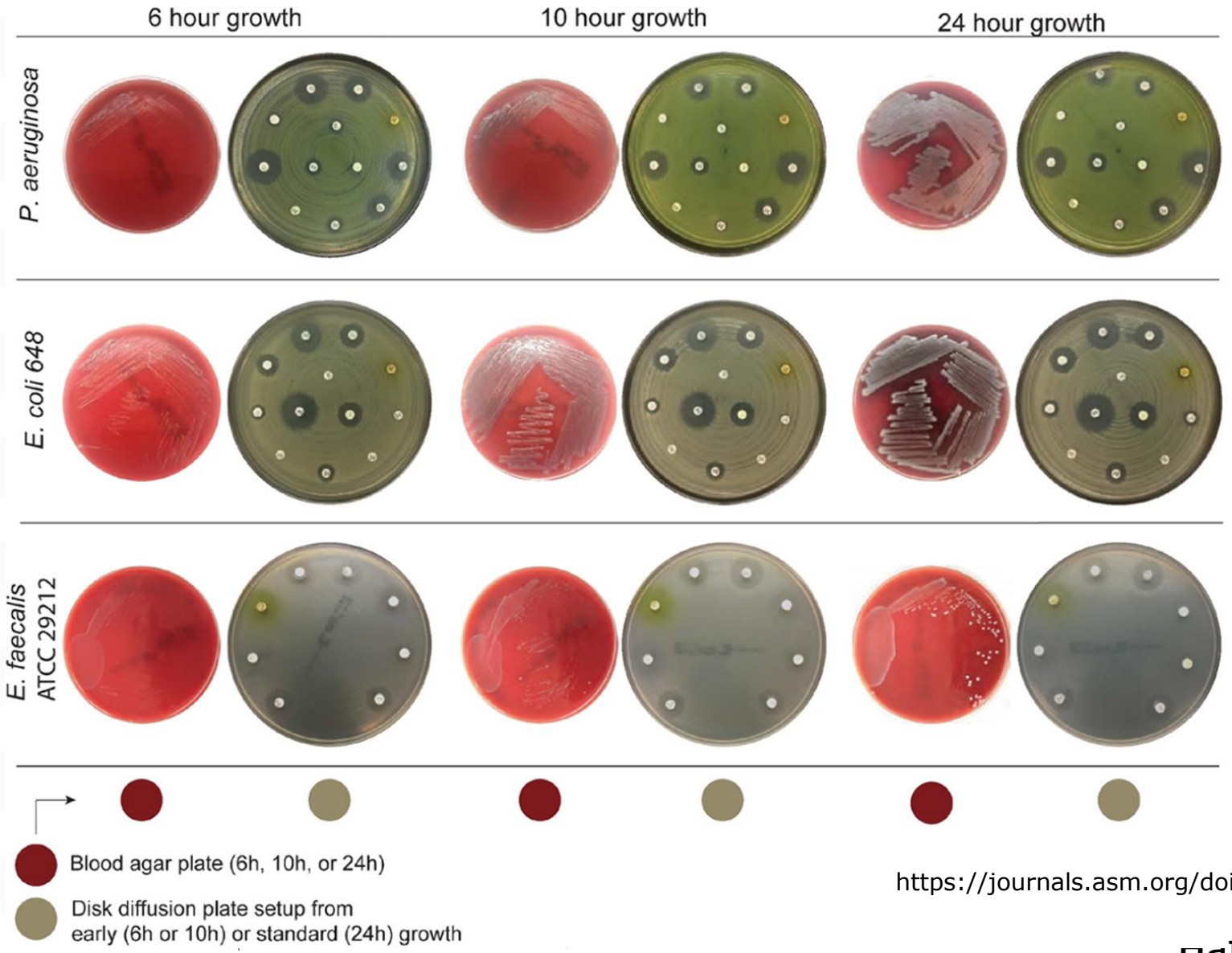
Disk Diffusion Performed on Early Growth



https://journals.asm.org/doi/epub/10.1128/jcm.03007-20

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Commercially Available Automated AST Platform

These are designed for testing pure isolates.

Microscan (Beckman Coulter)

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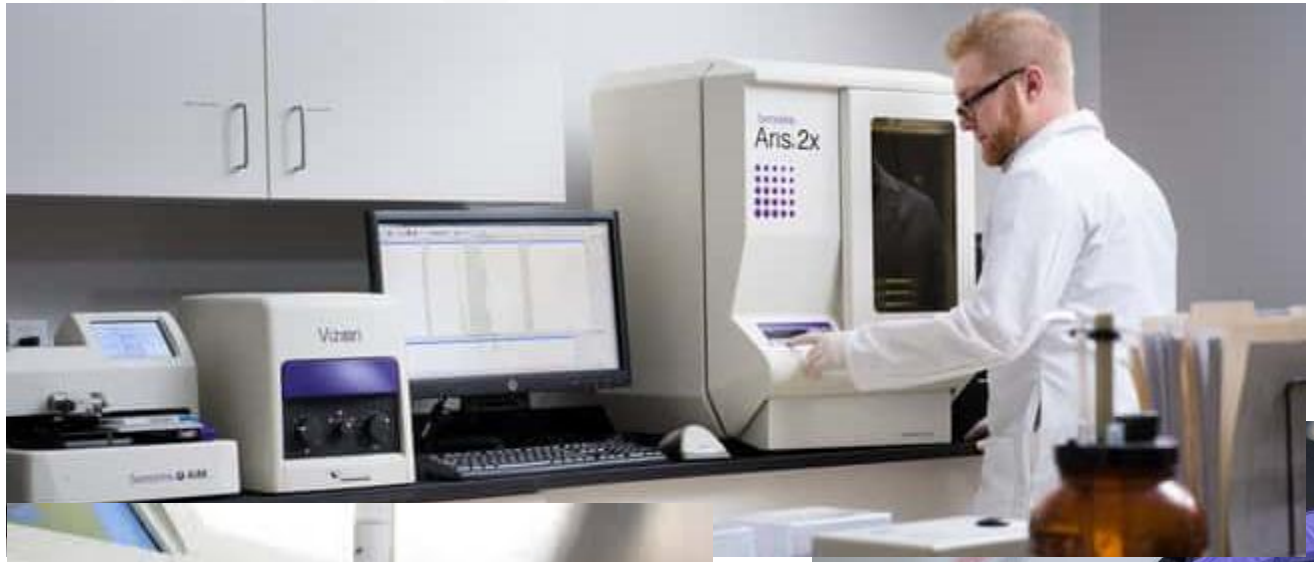
Sensititre (Thermo Scientific)



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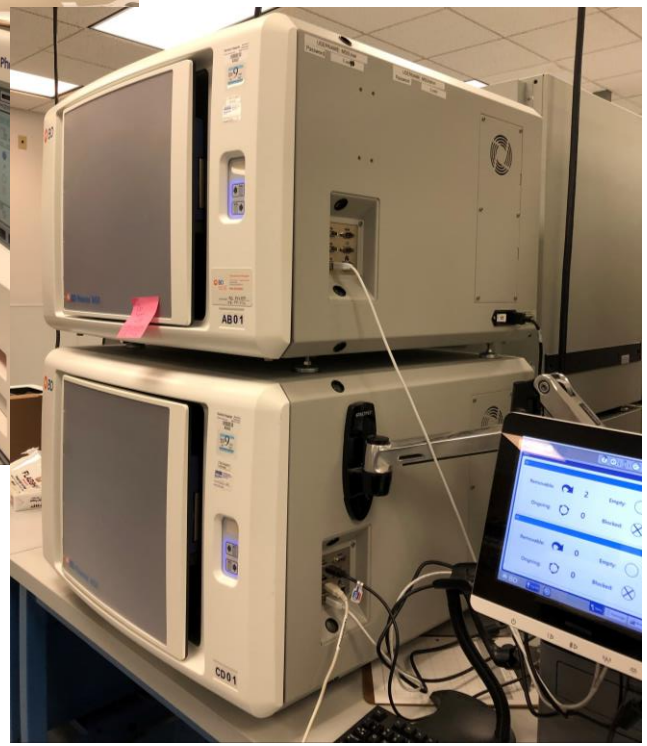
Phoenix AST (BD)



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ID/AST Combo Panel versus AST only panels

- End users should be aware of which antibiotics and concentration ranges are present on the panel and collaborate with antimicrobial stewardship for panel selection.
- ID/AST Combo panels will typically have less antibiotics and less concentrations of each.
- End users should be aware of antibiotic concentrations present per panel in order to appropriately assess for breakpoint changes.
- Panels will have exceptions for certain antibiotics and organisms, which will require an alternative testing method.

Clinical Cases: AMR

Using Kahoot!®

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CASE 1: part A

- 86 yr old male. Urine Culture.
- Growth: 10,000 CFU/mL of *Citrobacter freundii* complex
- AST Results via Phoenix NMIC-306 Panel: CPO well positive

Antibiotic	Interpretation	Antibiotic	Interpretation
Amoxicillin/ Clavulanate	Resistant	Ertapenem	Intermediate
Ampicillin	Resistant	Gentamicin	Resistant
Ampicillin/ Sulbactam	Resistant	Levofloxacin	Resistant
Cefazolin	Resistant	Meropenem	Susceptible
Cefepime	Susceptible dose dependent	Nitrofurantoin	Susceptible
Ceftazidime	Resistant	Tetracycline	Susceptible
Ceftriaxone	Resistant	Tobramycin	Intermediate
Ciprofloxacin	Resistant	Trimethoprim/Sulfamethoxazole	Susceptible

If you obtained either an intermediate or resistant ertapenem value, what would you do next?

A. Release AST reporting as Carbapenemase-producing organism.

B. Repeat initial AST panel to confirm result.

C. Perform alternative AST method for ertapenem and/or meropenem, i.e. by MIC strip (ETEST or MTS) or KB disk.

D. Perform mCIM

E. Perform CarbaNP

F. Perform Carba-R or similar molecular method

AST confirmation:

Citrobacter freundii complex

- Performed Ertapenem ETEST (0.38 µg/mL) SS.
- Carba-R (CREPCR) detected: KPC.
- Reported: This organism is a PCR confirmed carbapenemase producer (KPC).

CT DPH confirmed: "This *Citrobacter freundii* complex is potentially harboring a carbapenemase with low activity. Infectious Diseases and/or Infection Control consult is highly recommended."

Escherichia coli ESBL

- Repeated Phoenix NMIC-306 Panel
- CPO well repeated positive.
- Ertapenem repeated resistant (2 µg/mL)
- Carba-R (CREPCR) detected: OXA-48.
- Reported: *E. coli* ESBL producer. This organism is a PCR confirmed carbapenemase producers (OXA-48).

CT DPH confirmed: "This *Escherichia coli* is potentially harboring a carbapenemase with low activity. Infectious Diseases and/or Infection Control consult is highly recommended."

Awareness:

- Potential for the molecular detection of a known resistance gene to not match a phenotypic interpretation.
- Molecular tests are often more sensitive than phenotypic tests.
- Some examples that can be added to direct from specimen reports of molecular detection methods for provider awareness:
  - An ESBL gene has been detected by molecular assay; this may not correlate with cephalosporin susceptibility patterns. Please refer to detailed susceptibility results when available and suggest consultation with Infectious Diseases for management.
  - The KPC carbapenemase gene has been detected by molecular assay. Suggest consultation with Infectious Diseases for management.

Carbapenemase

- Enterobacterales that test resistant to at least one of the carbapenem antibiotics (ertapenem, meropenem, doripenem, or imipenem) or produce a carbapenemase (an enzyme that can make them resistant to carbapenem antibiotics) are called CRE.
- Carbapenemase-producing CRE make enzymes called carbapenemases that inactivate carbapenems and other β-lactam antibiotics, including penicillins and cephalosporins.
- *Klebsiella pneumoniae* carbapenemase (KPC)
- New Delhi Metallo-beta-lactamase (NDM)
- Verona Integron-Encoded Metallo-beta-lactamase (VIM)
- Imipenemase (IMP)
- Oxacillinase-48 (OXA-48)

Supplemental AMR Detection Assays: GNR

- Carbapenemase detection:
  - Phenotypic: CarbaNP; mCIM w/o eCIM; CPO well Phx; RAPIDEC CARBA NP
  - Molecular: Carba-R RT-PCR; RUO kits; syndromic panels; NGS

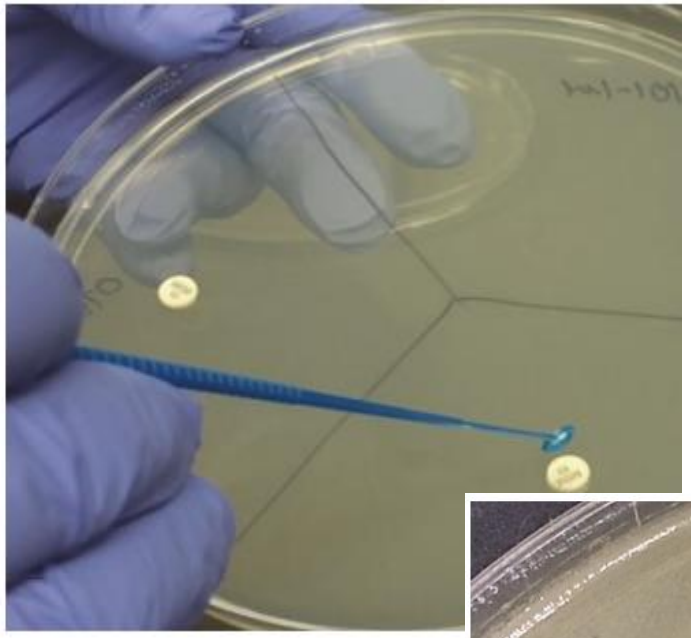
Supplemental Test	Organisms	Test Description	Optional for:	Table Locations
ESBL	• *E. coli* • *K. pneumoniae* • *Klebsiella oxytoca* • *Proteus mirabilis*	Broth microdilution or disk diffusion clavulanate inhibition test for ESBLs	Isolates demonstrating reduced susceptibility to cephalosporins Results that indicate presence or absence of ESBLs	3A
CarbaNP	• Enterobacterales • *P. aeruginosa*	Colorimetric assay for detecting carbapenem hydrolysis	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	3B, 3B-1
mCIM with or without eCIM	• mCIM only: Enterobacterales and *P. aeruginosa* • mCIM with eCIM: Enterobacterales only	Disk diffusion for detecting carbapenem hydrolysis (inactivation) eCIM add-on enables differentiation of metallo-ß-lactamases from serine carbapenemases in Enterobacterales isolates that are positive for mCIM	Isolates demonstrating reduced susceptibility to carbapenems Results that indicate presence or absence of certain carbapenemases	3C

Modified Carbapenem Inactivation Method: mCim

A

B

C



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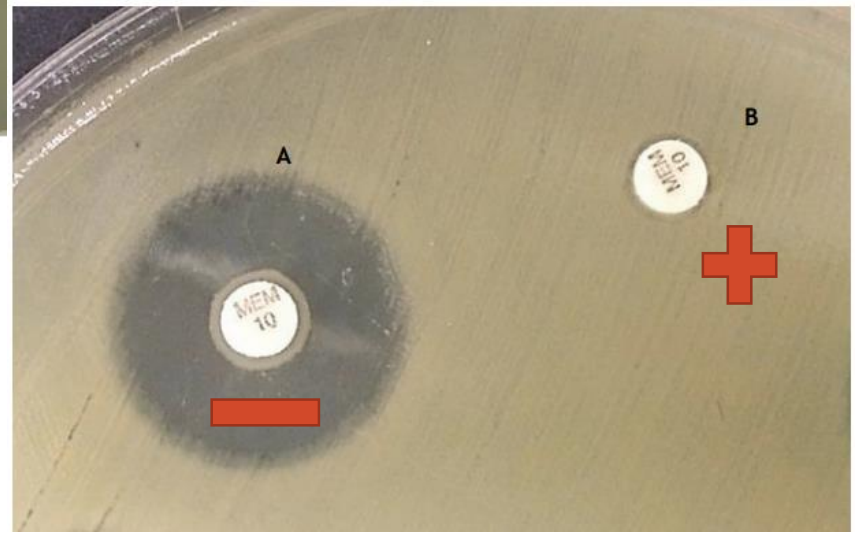

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eCIM: pair with mCIM to differentiate metallo-B-lactamases from serine carbapenemases.

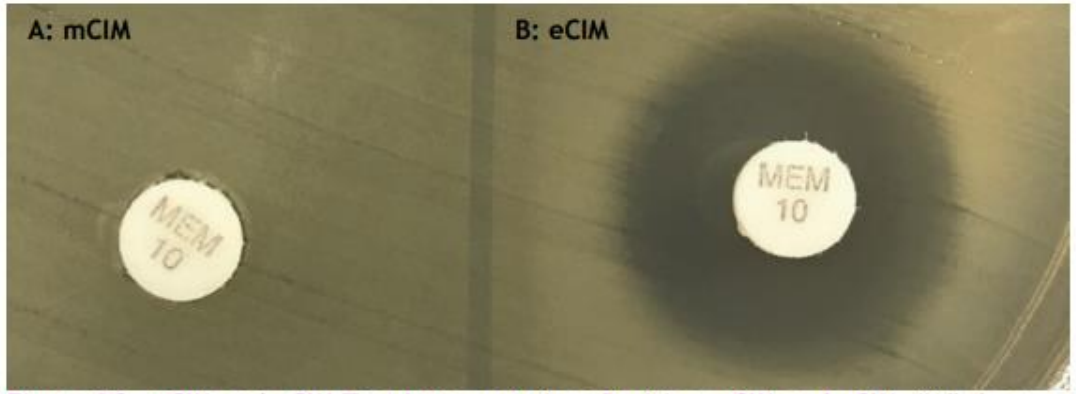

Figure 3C. mCIM and eCIM Test Interpretation: Positive mCIM and eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM positive result (zone diameter = 19 mm). A ≥ 5-mm increase in zone diameter for eCIM vs diameter for mCIM zone (19 mm – 6 mm = 13 mm) demonstrates the inhibition of the metallo-B-lactamase in the presence of EDTA.

- Result: positive mCIM and eCIM
- Report: metallo-B-lactamase detected

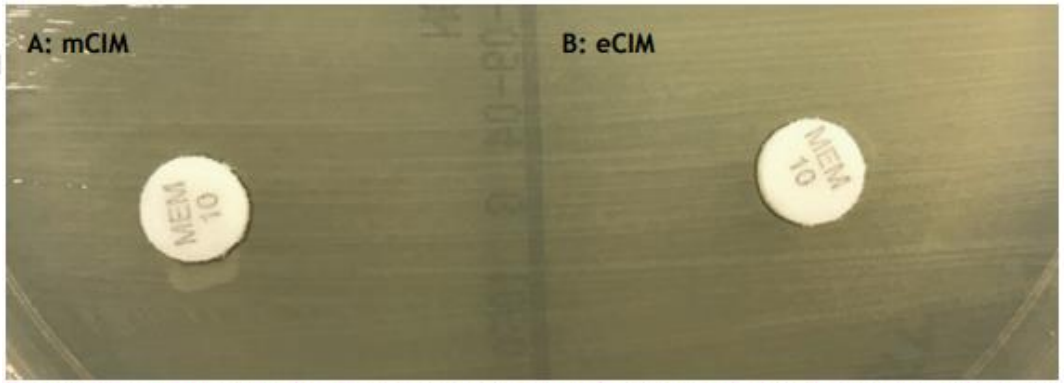

Figure 3D. mCIM and eCIM Test Interpretation: Positive mCIM and Negative eCIM. "A" shows an mCIM positive result (zone diameter = 6 mm) and "B" shows an eCIM negative result (zone diameter = 6 mm). Serine carbapenemases are not inhibited by EDTA and demonstrate a ≤ 4-mm increase in zone diameter for eCIM vs zone diameter for mCIM.

- Result: positive mCIM and negative eCIM
- Report: serine carbapenemase detected

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CarbaNP: may not detect OXA-48-like

- Utilize for isolates suspicious for carbapenemase production especially if new breakpoints are not implemented.

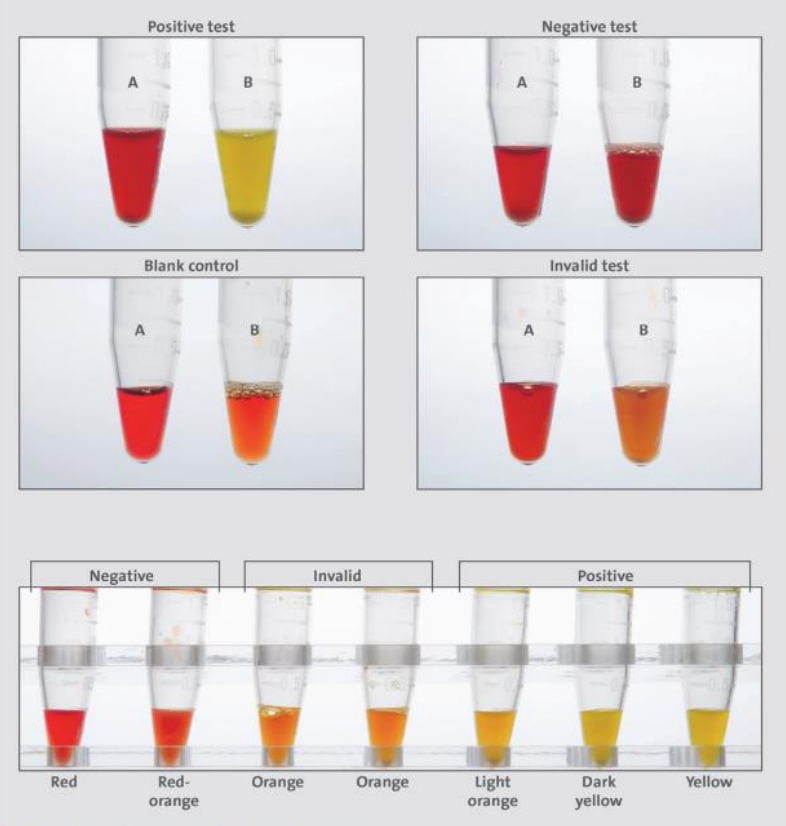

Figure 1. Interpretation of Color Reactions

Results for Patient and QC Tubes		
Tube "a": Solution A (serves as internal control)	Tube "b": Solution B	Interpretation
Red or red-orange	Red or red-orange	Negative, no carbapenemase detected
Red or red-orange	Light orange, dark yellow, or yellow	Positive, carbapenemase producer
Red or red-orange	Orange	Invalid
Orange, light orange, dark yellow, or yellow	Any color	Invalid

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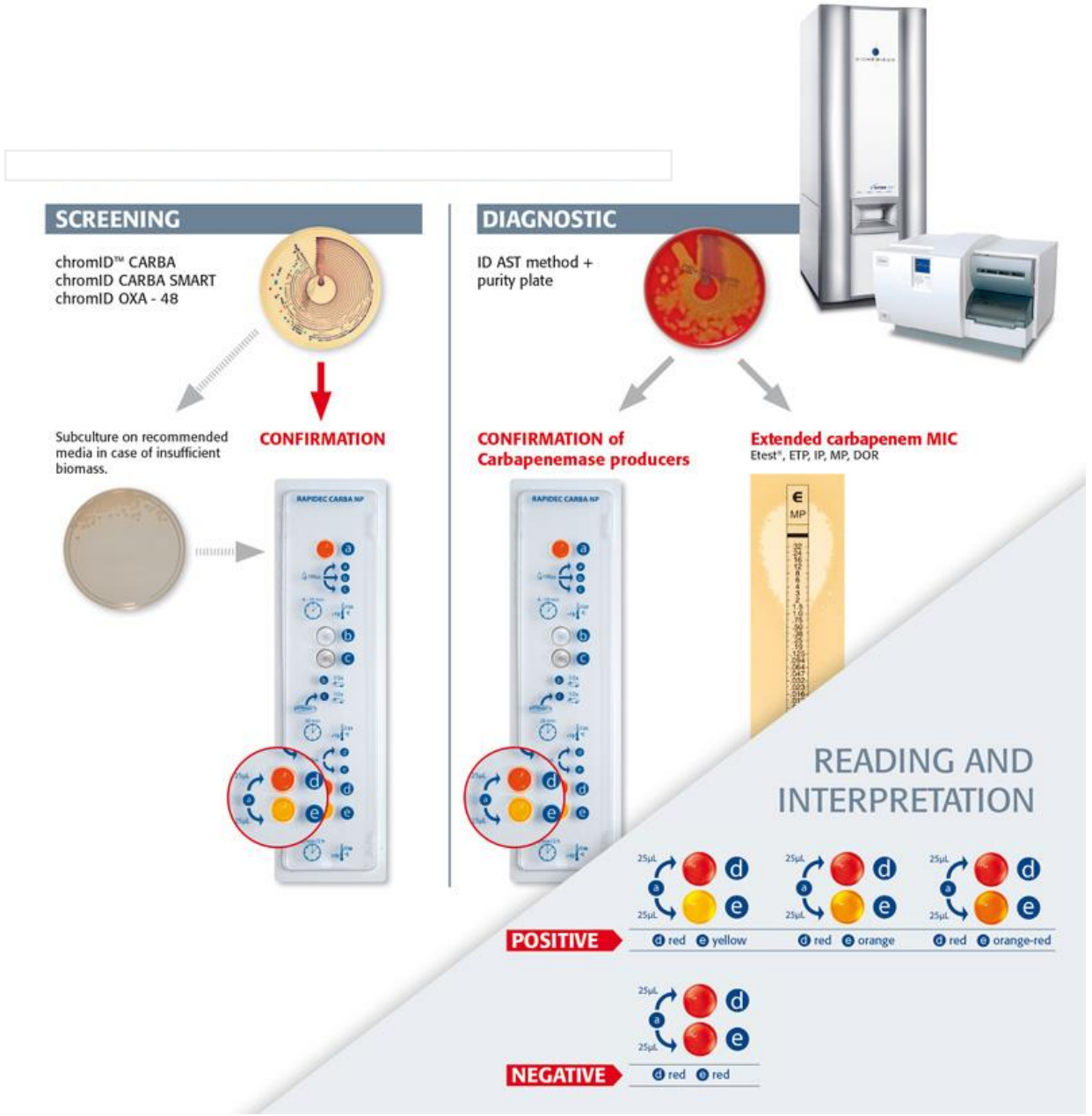
RAPIDEC CARBA NP

1) **Phenol red**: pH indicator

2) A **carbapenem**: imipenem (carbapenemase substrate) + **Zinc**, required for the detection of metallodependent carbapenemase-producing strains



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Molecular Options:

BD MAX™ Check-Points CPO assay is an integrated molecular screening solution

- Provides results for the five most common carbapenemase genes in Gram-negative bacteria
- Test can be processed from rectal swabs

KPC	OXA-48*	NDM	VIM/IMP

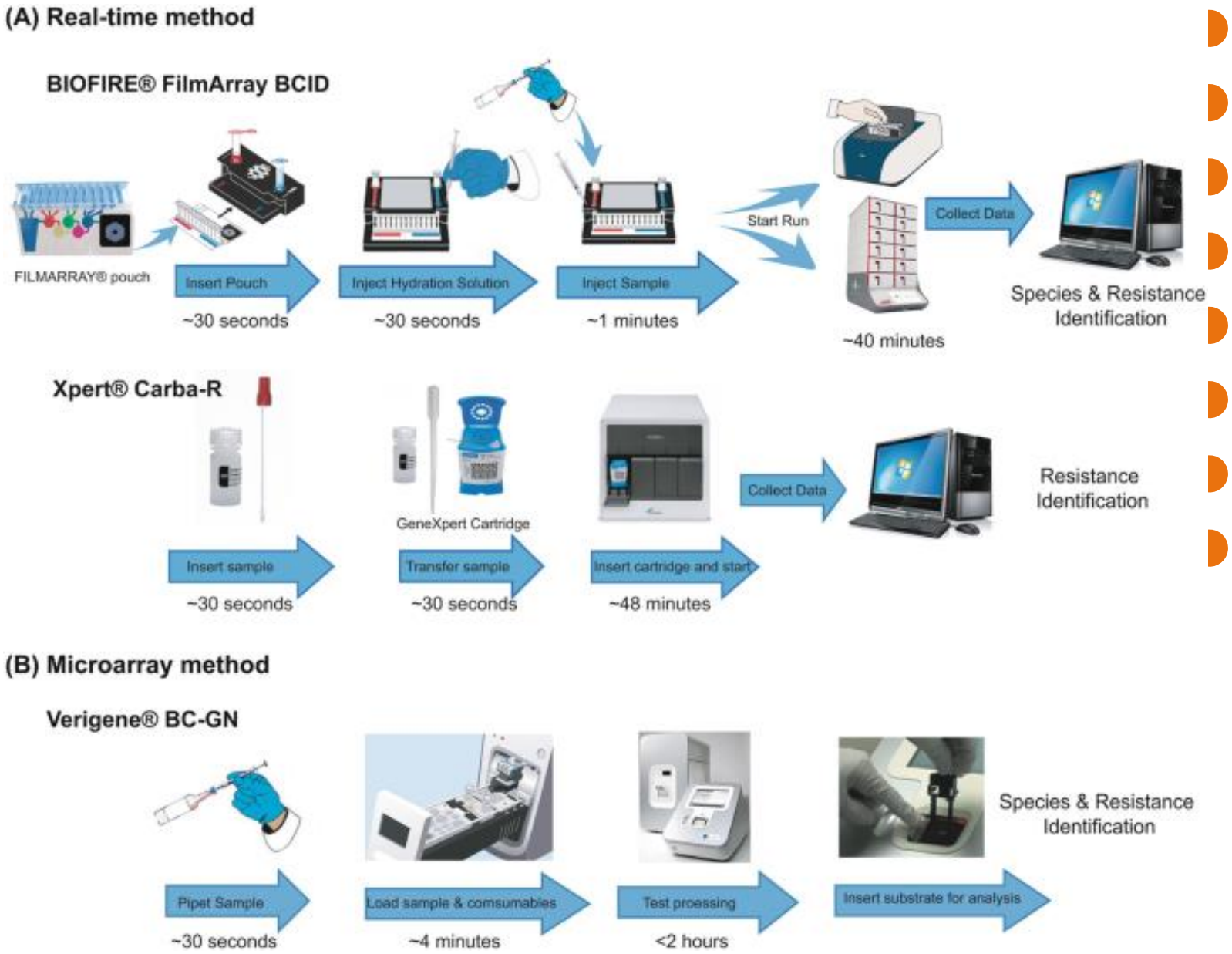
* OXA-48 and OXA-48 like

- Results in <2.5 hours compared to culture which can take up to 48 hours
- Fully integrated on the BD MAX™ System, a fully automated molecular system capable of running BD and partner developed assays as well as laboratory-developed tests

Improved patient management

Workflow efficiency

Fast screening results



Niu, S., Chen, L. (2018). https://doi.org/10.1007/978-3-319-95111-9_6

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RT-PCR options in research use only category.

illumina

Sequencing

For Research Use Only

DNA

AmpliSeq for Illumina Antimicrobial Resistance Research Panel

This sequencing research panel targets 478 antimicrobial resistance genes to help understand antibiotic treatment efficacy for 28 antibiotic classes.

HDPCR™ Multi-Drug Resistance Panel: For research use only. Not for use in diagnostic procedures.

HDPCR MDR	
Extended Spectrum Beta-Lactamases	
• CTX-M	
Carbapenemases	
• IMP	• OXA-48
• KPC	• VIM
• NDM	
Polymyxin Resistance	
• *mcr-1*	
Vancomycin Resistance (Enterococci)	
• *vanA*	
Toxigenic *C. difficile*	
• tcdB (*C. difficile* toxin B gene)	

ChromaCode

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What criteria is utilized by your DPH for submission of carbapenemase producing organisms?

A. Submit all Enterobacterales isolates with phenotypic resistance to at least one carbapenem and/or PCR detection of a carbapenemase gene (i.e. KPC, NDM, VIM, IMP, OXA-48).

B. Submit (5) *Psuedomonas aeruginosa* isolates resistant to meropenem or PCR detection of a carbapenemase gene.

C. Submit all *Acinetobacter* species isolates that are resistant to any carbapenem, except ertapenem, or PCR detection of a carbapenemase gene.

D. All of the above.

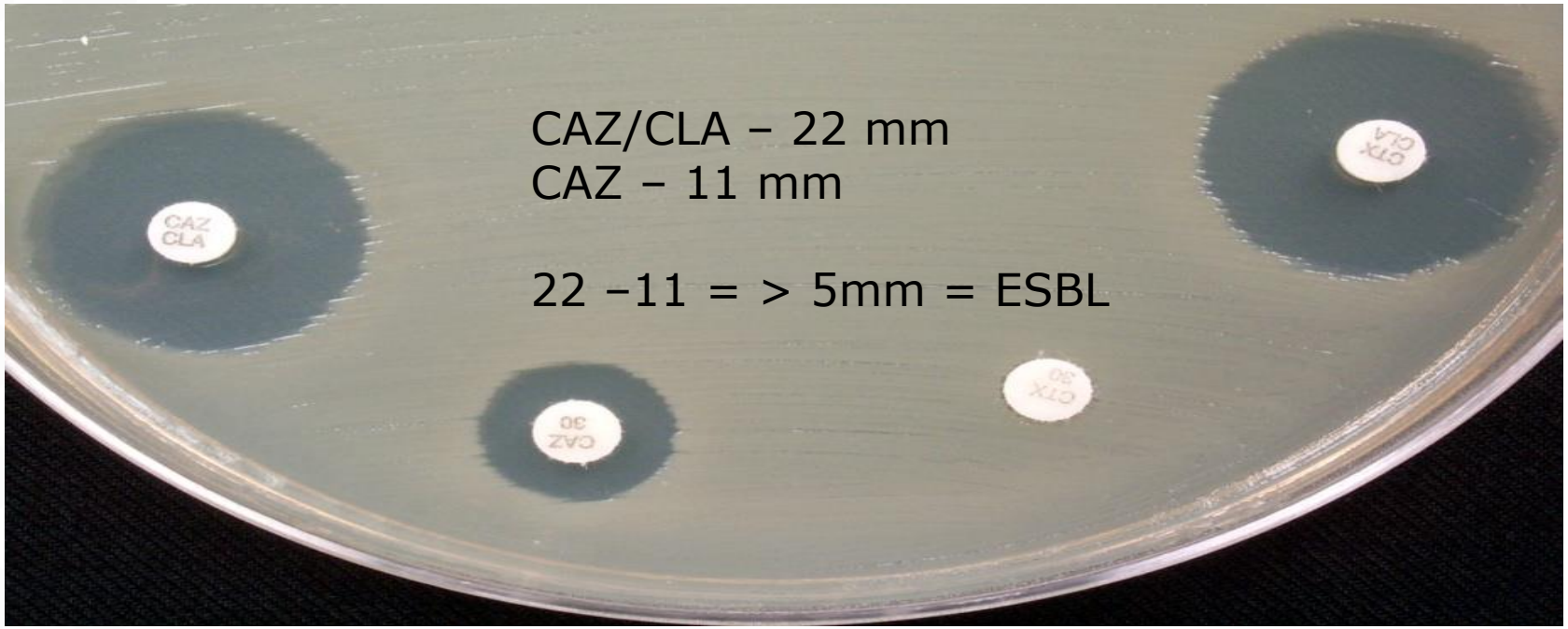
E. Other

Does your laboratory perform ESBL confirmation?

- Yes
- No
- Unsure

Extended-spectrum B-lactamase (ESBL)

- *Klebsiella pneumonia, Klebsiella oxytoca, Escherichia coli,* and *Proteus mirabilis.*
- Various ESBL genes are defined: CTX-M, SHV, TEM
- If current breakpoints are in use, then routine ESBL testing is not necessary but may be useful for epidemiological and infection prevention purposes.



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Case 2:

- 49 year old, female patient.
- Presented with worsening respiratory conditions (respiratory failure). Ventilator dependent. Various hospital stays and a trach for 4 months as the time of culture. Complicated medical history.
- Specimen: Lukens trap collection container for Respiratory Culture.
  - Gram stain: many neutrophils but no organisms seen.
  - Culture grew *Pseudomonas aeruginosa.*

Antibiotic	MIC (µg/mL)	Interpretation	Antibiotic	MIC (µg/mL)	Interpretation
Amikacin	>32	Resistant	Gentamicin	>8	Resistant
Ceftazidime	>16	Resistant	Levofloxacin	>4	Resistant
Cefepime	>16	Resistant	Meropenem	8	Resistant
Ciprofloxacin	>2	Resistant	Piperacillin / Tazobactam	>64/4	Resistant
Tobramycin	>8	Resistant	Ceftazidime / Avibactam	>8/4	Resistant
Ceftolozane / Tazobactam	>8/4	Resistant	CPO Well		Positive

M100-S32: Appendix B. Intrinsic Resistance

B2. Non-Enterobacterales

Organism \ Antimicrobial Agent	Ampicillin, Amoxicillin	Piperacillin	Ticarcillin	Ampicillin-sulbactam	Amoxicillin- clavulanate	Piperacillin-tazobactam	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Aztreonam	Imipenem	Meropenem	Ertapenem	Polymyxin B Colistin	Aminoglycosides	Tetracyclines/ Tigecycline	Trimethoprim	Trimethoprim-sulfamethoxazole	Chloramphenicol	Fosfomycin
Acinetobacter baumannii/ Acinetobacter calcoaceticus complex	R				R						R			R				R		R	R
Burkholderia cepacia complex[a]	R	R	R	R	R	a	a	a		a	a	a		R	R	a		a			R
Pseudomonas aeruginosa	R			R	R		R	R						R			R	R	R	R	
Stenotrophomonas maltophilia	R	R	R	R	R	R	R	R			R	R	R	R		R	[b]	R			R

Abbreviation: MIC, minimal inhibitory concentration; R, resistant.

NOTE 1: These nonfermentative gram-negative bacteria are also intrinsically resistant to penicillin (ie, benzylpenicillin), cephalosporins I (cephalothin, cefazolin), cephalosporin II (cefuroxime), cephamycins (cefoxitin, cefotetan), clindamycin, daptomycin, fusidic acid, glycopeptides (vancomycin), linezolid, macrolides (erythromycin, azithromycin, clarithromycin), quinupristin-dalfopristin, and rifampin.

NOTE 2: Information in boldface type is new or modified since the previous edition.

Case 2: *Pseudomonas aeruginosa*

- CPO well positive.
- Purity plate was not mixed.
- PHX GN Panel was repeated and susceptibility results remained the same.
- Carba-R PCR was performed: VIM was detected.
- Reported and confirmed: *Pseudomonas aeruginosa*. This organism is a PCR confirmed carbapenemase producer (VIM).
- This was an extremely resistant isolate.

CLSI M39-ED5:2022 Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data, 5th Edition

If you perform molecular based identification of resistance genes, are these results incorporated into the antibiogram?

A. Yes

B. No

C. Unsure

D. Molecular not performed

Table 24. Common Antimicrobial Resistance Genes and Predicted Phenotype Based on Presence and/or Absence of the Gene

Resistance Gene	Organisms	Phenotype Predicted and Accuracy of Prediction[a]			
		Presence	Approximate Accuracy	Absence	Approximate Accuracy
mecA	*S. aureus*	MRSA	98% to 100%	MSSA	98% to 100%
vanA/vanB	*E. faecalis* or *E. faecium*	VRE	95% to 100%	VSE	95% to 100%
bla_{CTX-M}	Enterobacterales *P. aeruginosa* *A. baumannii* *S. maltophilia*	ESBL producing gram-negative organism: resistance to penicillins, narrow- and expanded-spectrum cephalosporins	95% to 100%	N/A. The lack of detection does not predict susceptibility because many other mechanisms can lead to resistance.	50% to 95%
bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}	Enterobacterales *P. aeruginosa* *A. baumannii*	Carbapenemase-producing gram-negative organism: resistance to penicillins, narrow- and expanded-spectrum cephalosporins, and carbapenems	95% to 100%	N/A. The lack of detection does not predict susceptibility because many other mechanisms can lead to resistance.	10% to 98%

Case 3:

- 43 yr old female.
- Drainage collected for wound culture (aerobic culture + Gram stain).
- Gram stain: Many neutrophils and gram-positive cocci
- Identification: *Staphylococcus aureus*
- **Preliminary Antibiotic Profile:**

Antibiotic	MIC (µg/mL)	Interpretation	Antibiotic	MIC (µg/mL)	Interpretation
Ampicillin / Sulbactam	8/4	Resistant	Trimethoprim / Sulfamethoxazole	DD	Resistant
Clindamycin	> 4	Resistant	Ceftaroline	> 8	Resistant
Erythromycin	> 4	Resistant	Vancomycin	> 32	Resistant
Minocycline	> 8	Resistant			
Oxacillin	> 4	Resistant			
Tetracycline	> 8	Resistant			

What would you do if you obtained a vancomycin resistant result for *Staphylococcus aureus?*

A. Release results

B. Check purity plate

C. Report to the public health laboratory

D. Unsure

E. Repeat AST from a fresh isolate

Case 3:

- 43 yr old female.
- Drainage collected for would culture (aerobic culture + Gram stain).
- Identification: *Staphylococcus aureus.*
- **Phx GP Panel repeated on a fresh isolate.**

Antibiotic	MIC (µg/mL)	Interpretation	Antibiotic	MIC (µg/mL)	Interpretation
Ampicillin / Sulbactam	≤ 2/1	Susceptible	Trimethoprim / Sulfamethoxazole	DD	Susceptible
Clindamycin	< 0.5	Susceptible	Ceftaroline	0.25	Susceptible
Erythromycin	> 4	Resistant	Vancomycin	1	Susceptible
Minocycline	≤ 1	Susceptible			
Oxacillin	> 4	Resistant			
Tetracycline	≤ 0.5	Susceptible			

D-Zone Test: Inducible Clindamycin Resistance

- Inducible Clindamycin Resistance: erythromycin and clindamycin
  - If erythromycin tests resistant but clindamycin tests susceptible or intermediate in *Staphylococcus* or certain *Streptococcus* species then perform a D-test to ensure that an inducible clindamycin resistance gene is not present.

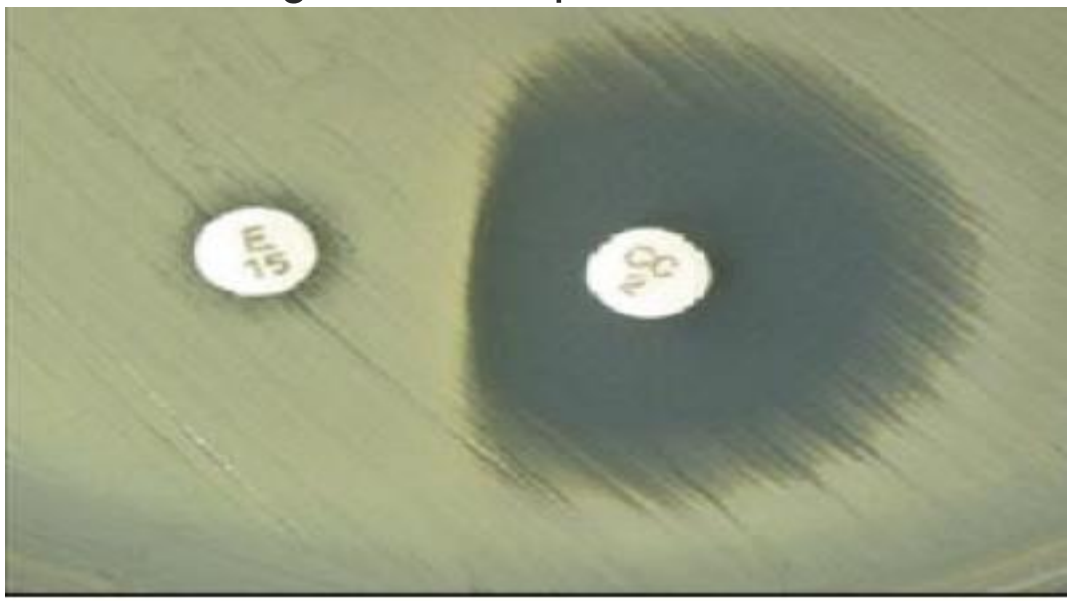

Figure 8.7—Blunting of clindamycin zone due to inducible resistance.

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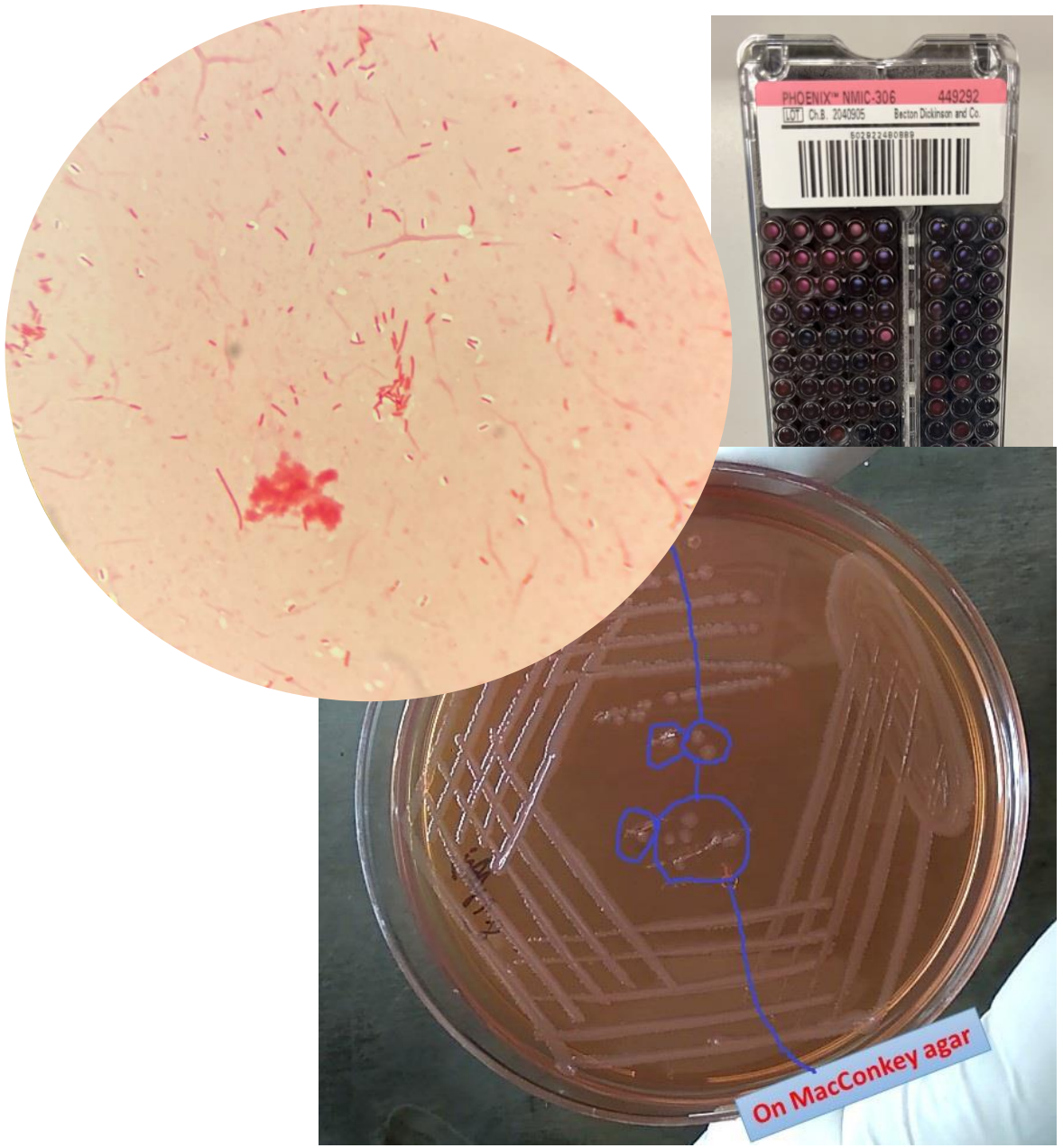
Case 3:

- D-zone Test positive: report clindamycin resistant.
- This isolate is oxacillin resistant, report Methicillin-Resistant *Staphylococcus aureus* (MRSA).
- Options also exist for molecular detection of MRSA through *mecA*, *mecC*, *mecA/C*, and *mecA/C* with MREJ cassette.

Case:4

- 8 year old patient
- Presented with fever, nausea, vomiting, and diarrhea.
- Recent travel to Pakistan (1 month duration).
- Blood Cultures positive: gram-negative rod.
- MALDI-TOF MS identification performed using Sepsityper process: *Salmonella* species
- Since this was an extraintestinal *Salmonella* antibiotic susceptibility testing was automatically performed using BD Phoenix NMIC-306 panel on pure isolated colony growth.
- Antibiotic susceptibility reporting is based on antibiotic cascade rules, which include CLSI M100S32 guidance.



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Does you laboratory automatically perform AST testing for *Salmonella* isolates?

- Yes, both intestinal and extraintestinal isolates.
- No, only by clinician request.
- Yes, extraintestinal isolates only
- Yes, but only for *Salmonella enterica* serotype Typhi

Case 4:

Antibiotic	MIC (mcg/mL)	Interpretation
Ampicillin	>16	Resistant
Cefepime	>16	Resistant
Ceftazidime	>16	Resistant
Ceftriaxone	>32	Resistant
Trimethoprim/Sulfamethoxazole	>2/38	Resistant
Ciprofloxacin	3	Resistant*no bp change
Levofloxacin	8	Resistant*no bp change

- Speciation performed by the CT DPH: *Salmonella enterica* serotype Typhi
- Extensively Drug Resistant (XDR) Typhoid Fever.
- Requested AST for Meropenem & Azithromycin.
- Treatment: Tigecycline initially then switched to 14 days Meropenem q8hrs IV via PICC.

Which of the following classes of antibiotics should not be reported as susceptible, even if AST results susceptible, since not clinically effective for *Salmonella*?

A. Cephalosporins I (cefazolin)

B. Cephalosporins II (cefuroxime)

C. Aminoglycoside (gentamicin)

D. Cephamycins (cefoxitin)

E. All of the above

M100-S32, Appendix B. Intrinsic Resistance Tables

Appendix B. (Continued)

B1. Enterobacterales (Continued)

Organism \ Antimicrobial Agent	Ampicillin	Amoxicillin-clavulanate	Ampicillin-sulbactam	Ticarcillin	Cephalosporins I: Cefazolin, Cephalothin	Cephamycins: Cefoxitin, Cefotetan	Cephalosporin II: Cefuroxime	Imipenem	Tetracyclines	Tigecycline	Nitrofurantoin	Polymyxin B Colistin	Aminoglycosides
Salmonella and *Shigella* spp.	There is no intrinsic resistance to ß-lactams in these organisms; refer to WARNING below for reporting.												
Serratia marcescens	R	R	R		R	R	R				R	R	
Yersinia enterocolitica	R	R		R	R								

Abbreviation: R, resistant.

WARNING: For *Salmonella* spp. and *Shigella* spp., aminoglycosides, first- and second-generation cephalosporins, and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

Direct from Positive Blood Culture/Rapid AST Options

Current and Future

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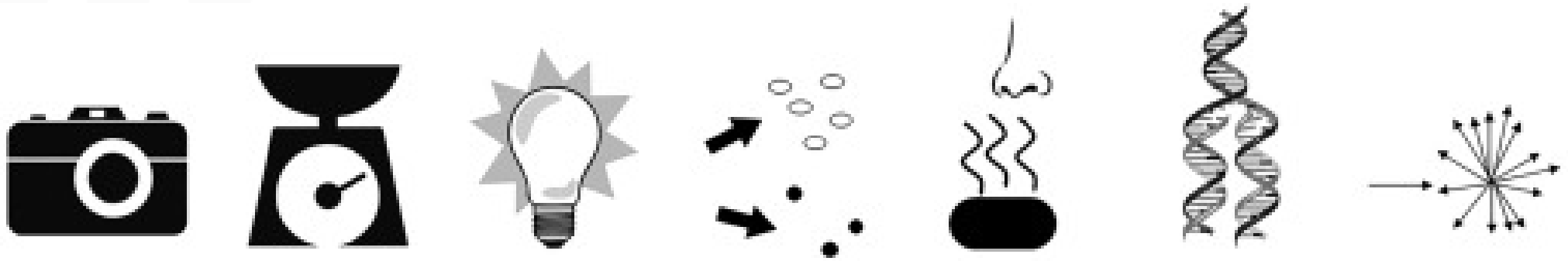
Snapshot of direct from specimen AST: current & in development



Time-lapse imaging	Mass measurement	Detect bioluminescence	Cell sorting / counting	Detect volatile compounds	Evaluation of transcription changes	Light scattering
Phenotest BC (Accelerate Dx) dRAST (QuantiMatrix) ASTar (Q-linea) NGP (SeLux) QuickMIC (Gradientech)	LifeScale (Affinity Biosensors)	IDAST (Draper) vivoDx (Roche)	FASTinov MultiPath (Firstlight Dx)	Reveal AST (Specific Diagnostics)	GoPhAST (NanoString)	Alfred (AliFAX) 216R (BacterioScan)

- CLSI Direct Disk Diffusion Testing
- Qvella FAST Prep Cartridge (pair with MALDI-TOF ID & validate on routine AST
- Pattern Bioscience

Romney. 2020. LabMed

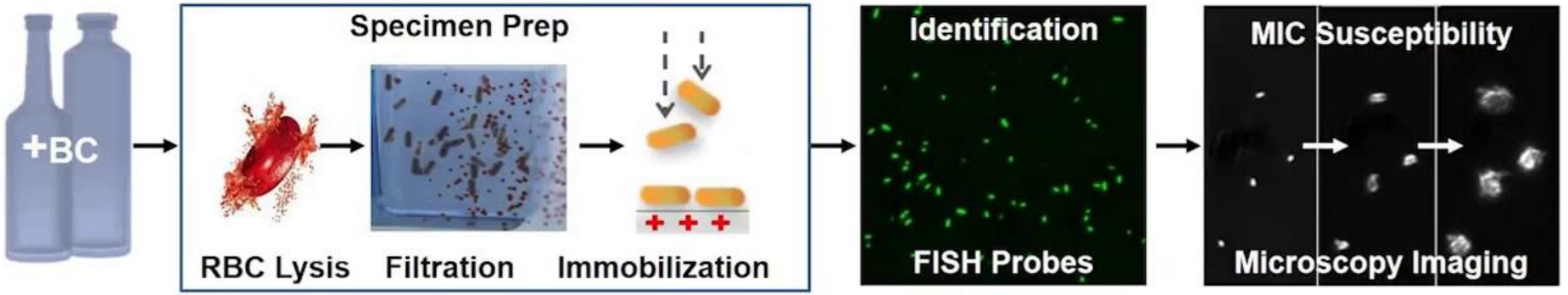
Direct from (+) Blood Culture Bottle: ID + AST

Accelerate Pheno™ System

System
- 1-4 module(s)
- Control & Analysis PCs
- Touchscreen monitor

Module
- Automated pipetting robot
- Digital camera
- Custom microscope

Kit
- 48 flow-channel cassette
- Reagent cartridge
- Sample vial



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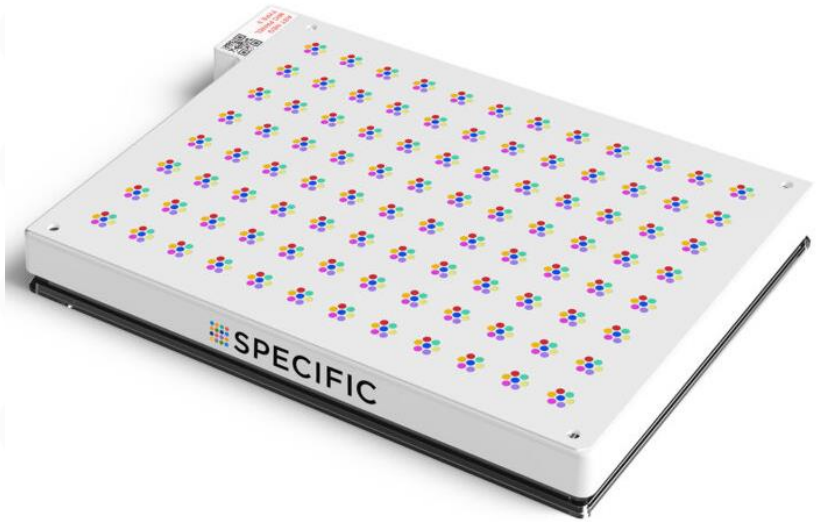
Sampling of direct from Specimen AST:



SPECIFIC

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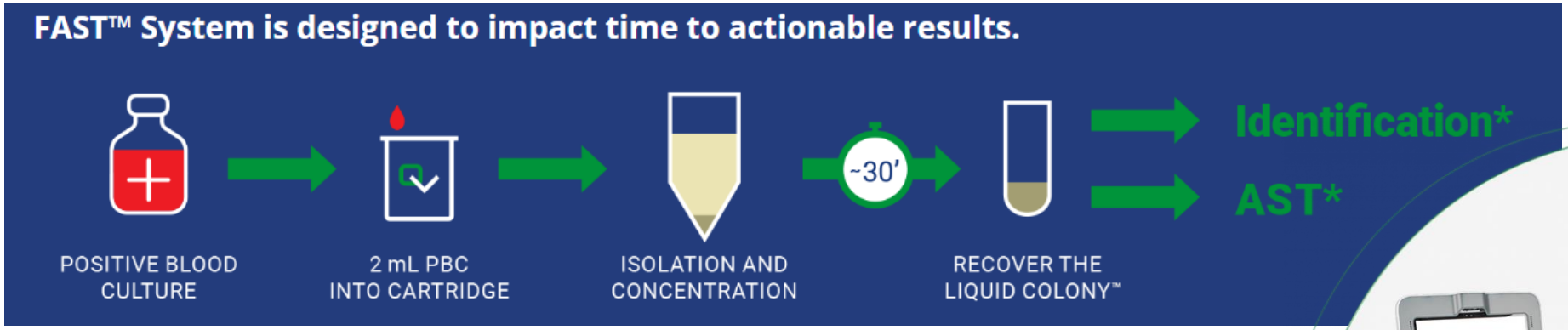
Sampling of direct from Specimen AST:

Qvella™
microbiology in minutes™

Class I IVD devices with the US FDA



- Device instead of AST panel.
- The objective is to pair with MALDI-TOF MS for rapid identification (if single morphotype), then use the same liquid colony to setup legacy AST.
- Laboratory would need to validate that the concentration obtained as a liquid colony performs comparable to standard inoculum for AST.

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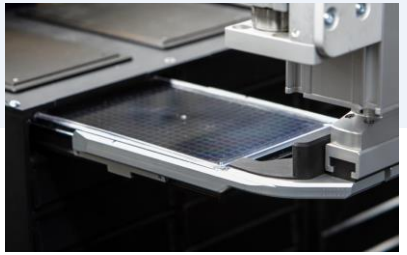
Sampling of direct from Specimen AST:

PBC Separator

Inoculator



Analyzer

Instrument in development not for clinical use

Next-Generation Phenotyping (NGP)

SeluxDX

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CLSI Method for Direct Disk Diffusion Testing From Positive Blood Cultures.

- Laboratory workflows for this method vary based on whether or not the organism identification is available at the time of direct DD set up.
- Direct DD must be set up within 8 hours of the blood culture bottle flagging positive for gram-negative bacilli.
- Identification at the time of direct DD set up include rapid molecular tests or direct from positive blood culture bottle matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

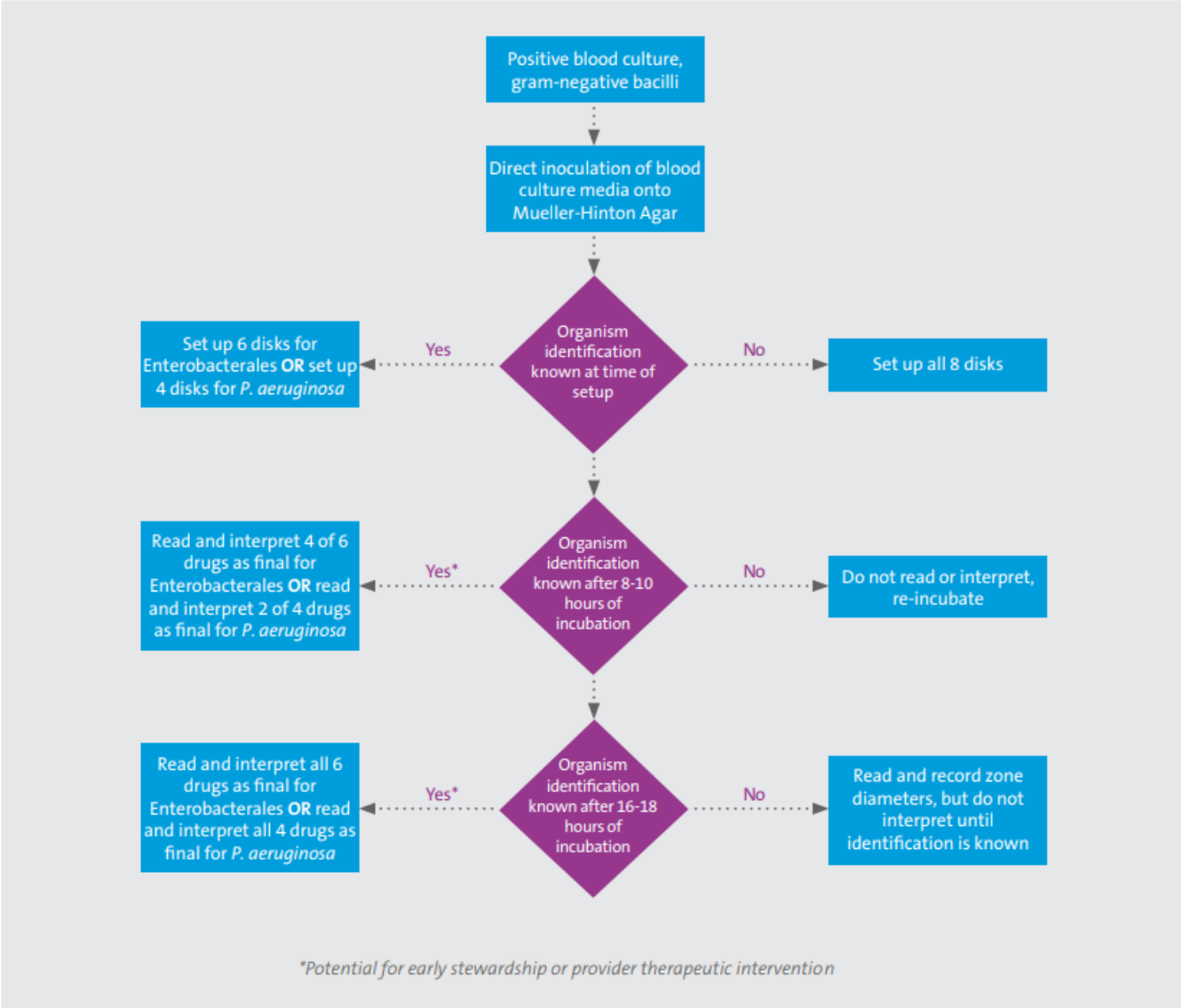

Figure 1. Workflow for direct blood culture disk diffusion testing

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CLSI Method for Direct Disk Diffusion Testing From Positive Blood Cultures

- To establish breakpoints, results from the direct DD method using positive blood culture broth as the inoculum with incubation of the DD test for 8-10 and 16-18 hours were compared to the standard DD method using isolated colonies with 16-18 hours incubation.
- Refer to M100S32 for breakpoints used for direct blood culture DD for Enterobacterales and *P. aeruginosa.*

Table 1. CLSI Breakpoints for Direct Blood Culture Disk Diffusion

Antimicrobial	Enterobacterales		*Pseudomonas aeruginosa*	
	8-10 hr read	16-18 hr read	8-10 hr read	16-18 hr read
Ampicillin		X		
Aztreonam	X[a]	X		
Ceftazidime	X[a]	X		X[a]
Ceftriaxone	X[a]	X		
Ciprofloxacin			X[a,b]	X[a]
Meropenem				X[a]
Tobramycin	X[a]	X	X[a]	X[a]
Trimethoprim-sulfamethoxazole		X		

[a] Antimicrobials with direct DD newly provided in M100 32nd edition.
[b] Antimicrobial for which direct DD breakpoints differ from the standard DD breakpoints.

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