ANTIMICROBIAL SUSCEPTIBILITY TESTING AND SURVEILLANCE: FROM LABORATORY TO CLINIC

DILUTION METHODS

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Antibiogram: Methods

1. **Phenotypic assays**

   (Direct bacteria-antimicrobial agent interaction)

   Diffusion methods: Disk assay

   Gradient diffusion (Etest® and others)

   Dilution methods:

   Broth (macro- & microdilution)

   Solid medium (agar)

   (Semi)-automatic methods

2. Detection of biochemical mechanisms of resistance

3. Detection of resistance genes

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MIC

The lowest concentration OF ANTIMICROBIAL AGENT (in mg/l) inhibiting visible growth of the tested microorganism under standard conditions.

MBC

The lowest concentration OF ANTIMICROBIAL AGENT (in mg/l) killing 99.9%* of the initial inoculum of the tested microorganism under standard conditions.

*** [99%?]**

A bacterium DOES NOT have an MIC… an antibiotic HAS!

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CMI ≈ CMB	**BACTERICIDAL DRUG**
CMI < CMB	**BACTERIOSTATIC DRUG**

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TOLERANCE

CMB>CMI (AT LEAST 16-32 TIMES)
FOR USUALLY BACTERICIDAL
ANTIMICROBIAL AGENTS

[BUT…. Difficulties to differentiate between Tolerance and Persistence!]

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PARADOXICAL EFFECT

DECREASED BACTERIAL DEATH IN INCREASING CONCENTRATIONS OF ANTIMICROBIAL AGENT

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DILUTION METHODS

ANTIMICROBIAL AGENTS

-Serial two-fold dilutions

-Pay attention to solvent and diluent, stability, and temperature of conservation of reference powders)

INOCULUM

- 10^5 CFU/ml (usually prepared from a suspension with a turbidity equivalent to McFarland 0.5)

MEDIUM

- Mueller Hinton agar/broth (cation-adjusted). Supplemented as necessary

-Haemophilus Test Medium; Others

INTERPRETATION

- MIC: absence of visible growth by the naked eye (usual incubation: 18h-35ºC)

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M07-A8
Vol. 29 No. 2
Replaces M07-A7
Vol. 26 No. 2

January 2009

Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition

This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

A standard for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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M45-A
Vol. 26 No. 19
Replaces M45-P
Vol. 25 No. 26

Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline

This document provides guidance to clinical microbiology laboratories for standardized susceptibility testing of infrequently isolated or fastidious bacteria that are not presently included in CLSI/NCCLS documents M2, M7, or M11. The tabular information in this document presents the most current information for drug selection, interpretation, and quality control for the infrequently isolated or fastidious bacterial pathogens included in this guideline.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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(Formerly NCCLS)

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M100-S20
Vol. 30 No. 1
Replaces M100-S19
Vol. 29 No. 3

January 2010

Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement

This document provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A10 and M07-A8.

An informational supplement for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Table 1. Suggested Groupings of U.S. FDA-Approved Antimicrobial Agents That Should Be Considered for Routine Testing and Reporting on Nonfastidious Organisms by Clinical Microbiology Laboratories

	Enterobacteriaceae[g]	*Pseudomonas aeruginosa*[j]	*Staphylococcus* spp.	*Enterococcus* spp.[n]
GROUP A PRIMARY TEST AND REPORT	Ampicillin[g]	Ceftazidime	Oxacillin[l]	Penicillin[o] or ampicillin
	Cefazolin[a] Cephalothin[a]	Gentamicin	Penicillin[l]	
	Gentamicin	Mezlocillin or ticarcillin Piperacillin		
GROUP B[e] PRIMARY TEST REPORT SELECTIVELY	Amikacin	Amikacin	Azithromycin[b] or clarithromycin[b] or erythromycin[b]	**Daptomycin[s]**
				Linezolid
	Amoxicillin-clavulanic acid or ampicillin-sulbactam Piperacillin-tazobactam Ticarcillin-clavulanic acid	Aztreonam Cefoperazone		Quinupristin-dalfopristin[r]
			Clindamycin[f]	Vancomycin[p]
			Daptomycin	
	Cefamandole or cefonicid or cefuroxime		Linezolid	
			Telithromycin[b]	
	Cefepime	Cefepime	Trimethoprim-sulfamethoxazole	
	Cefmetazole Cefoperazone[g] Cefotetan Cefoxitin	Ciprofloxacin Levofloxacin	Vancomycin	
	Cefotaxime[g, h, i] or ceftizoxime[g, i] or ceftriaxone[g, h, i]	Imipenem Meropenem		
	Ciprofloxacin[g] or levofloxacin[g]	Tobramycin		
	Ertapenem Imipenem or meropenem			
	Mezlocillin or piperacillin Ticarcillin			
	Trimethoprim-sulfamethoxazole[g]			
GROUP C[t] SUPPLEMENTAL REPORT SELECTIVELY	Aztreonam Ceftazidime (Both are helpful indicators of extended-spectrum β-lactamases.)[i]	Netilmicin	Chloramphenicol[b]	Gentamicin (high-level resistance screen only)
	Chloramphenicol[b, g]		Ciprofloxacin or levofloxacin or ofloxacin Gatifloxacin **or moxifloxacin**	Streptomycin (high-level resistance screen only)
	Kanamycin		Quinupristin-dalfopristin[m]	
	Netilmicin		Gentamicin	Chloramphenicol[b] Erythomycin[b] Tetracycline[c] Rifampin[d] (These agents may be tested for VRE)[q]
	Tetracycline[c]		Rifampin[d]	
	Tobramycin		Tetracycline[c]	
GROUP U SUPPLEMENTAL FOR URINE ONLY	Carbenicillin	Carbenicillin	Lomefloxacin or norfloxacin	Ciprofloxacin Levofloxacin Norfloxacin
	Cinoxacin Lomefloxacin or norfloxacin or ofloxacin	Lomefloxacin or norfloxacin or ofloxacin	Nitrofurantoin	Nitrofurantoin
	Gatifloxacin			
	Loracarbef		Sulfisoxazole	Tetracycline[c]
	Nitrofurantoin			
	Sulfisoxazole			
	Trimethoprim		Trimethoprim	

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TABLA 1. Antibióticos y concentraciones en la determinación de la sensibilidad de los microorganismos Gram-negativos

Antimicrobianos		Concentraciones (µg/ml)	Enterobact.	*P. aerug.*	*Acinet.*	Otros
ß-lactámicos	Ampicilina[a]	32-**16-8-4**-2	A1			
	Amoxicilina/clav.	32/16-**16/8-8/4-4/2**	A1			
	Ampicilina/sulb.	32/16-**16/8-8/4-4/2**			A2	
	Ticarcilina	**128-64-32**-16		A1	A1	A1
	Piperacilina	128-**64-32-16**-8	C3			
	Piper./tazob.	128/4-**64/4-32/4-16/4**-8/4	A1	A1	A1	A1
	Cefazolina	32-**16-8**-4-2	A1			
	Cefuroxima	32-**16-8-4**-2-1	A1			
	Cefoxitina	32-**16-8**-4	A2			
	Cefotetan	**32-16**	C3			
	Ceftazidima	32-**16-8-4-2-1**-0,5	A1	A1	A1	A1
	Ceftazidima/clav.	**8/4**-4/4-2/4-**1/4**	B2 **[6]**			
	Cefotaxima	**32-16-8-4-2-1**-0,5	A1			
	Cefotaxima/clav.	**8/4**-4/4-2/4-**1/4**	B2 **[6]**			
	Cefepima	32-**16-8-4-2-1**-0,5	A1	A1	A1	A1
	Cefepima/clav.	**8/4**-4/4-2/4-**1/4**	B2			
	Aztreonam	32-**16-8-4-2-1**-0,5-0,25-0,12	A1	A2	A2	A2
	Imipenem	16-**8-4-2-1**-0,5-0,25-0,12 **[9]**	A1	A2	A1	A1
	Meropenem **[10]**	16-**8-4-2-1**-0,5-0,25-0,12 **[9]**		A1	A2	A2
	Ertapenem	16-**8-4-2-1**-0,5-0,25-0,12 **[9]**	B2			
Aminoglicósidos	Gentamicina	**8-4-2**	A1	B2	B2	B2
	Tobramicina	**8-4-2**	B2	A1	A1	A1
	Amicacina	64-**32-16-8**-4	A2	A1	A1	A1
	Netilmicina	**8-4-2**			B2	
Nitrofuranos	Nitrofurantoína	128-**64**-32	A4			
Quinolonas	Ac. nalidíxico	32-**16**-8-4	A2			
	Norfloxacino **[12]**		A4[b]			
	Ciprofloxacino	4-**2-1-0,5-0,25-0,12**	A1	A1	A1	A1
	Levofloxacino	8-**4-2-1-0,5-0,25-0,12**				A2
Tetraciclinas	Tetraciclina	16-8-**4**-2-1-0,5-0,25	C3			
	Minociclina	**16-8-4**-2-1-0,5-0,25				A1
	Tigeciclina	8-4-**2-1-0,5**-0,25-0,12	A1		A1	A2
Otros	Ac. pipemídico	**[13]**	A4			
	Cotrimoxazol	**4/76-2/38**	A2, A4			A1
	Fosfomicina	256-128-**64-32-16**-8	A2, A4	A2	A2	A2
	Cloranfenicol	32-16-**8**-4	C3			
	Colistina	8-**4-2**-1	C3	A2	A2	A2

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Organisms	Microdilution	Agar dilution	Tº	Incubation time
Enterobacteria *P. aeruginosa* GNB non-Enterobact. *Vibrio choleare*	CAMHB	MHA	35±2ºC	16-20h
Acinetobacter spp. *Burkholderia cepacia* *Stenotrop. maltophila*	CAMHB	MHA	35±2ºC	24-24h
Staphylococcus	CAMHB CAMHB+2%ClNa(OXA) CAMHB+50mg/l (DAP) Screening: Other media	MHA MHB+2%ClNa(OXA)	35±2ºC	16-20h (24h, OXA 24 h, VAN)
Enterococcus	CAMHB CAMHB+50mg/l (DAP) BHIB: VAN/HLAR Screen	MHA BHIA: VAN/HLAR Screen	35±2ºC	16-20h (24h, OXA 24 h, VAN)
Haemoph. influenzae; *H. parainfluenzae*	HTM broth		35±2ºC	24-24h

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Organisms	Microdilution	Agar dilution	Tº	Incubation time
Streptococcus pneumoniae	CAMHB+LHB		35±2ºC	20-24h
Streptococcus viridans beta-hemolytic	CAMHB+LHB CAMHB+LHB+Ca (DAP)	HMA+ShB (LHB)	35±2ºC	20-24h
Neisseria gonorrhoeae		GC+Supplement	36±1ºC	20-24h
Neisseria meningitidis	CAMHB+LHB	HMA+ShB	35±2ºC	20-24h
Helicobacter pylori		MHA+aged SB	35±2ºC	3 days

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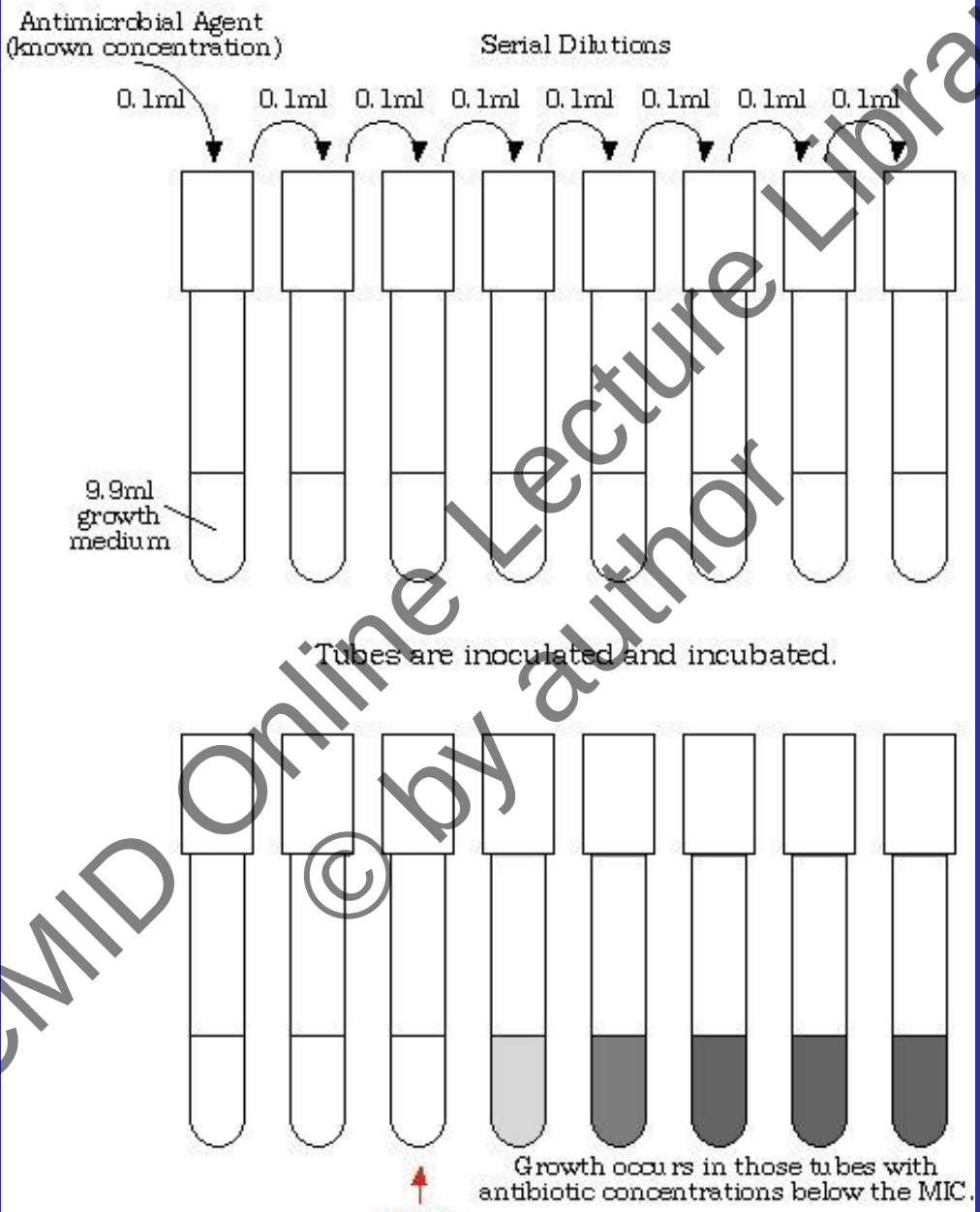
Determination of the MIC: Tube Dilution Assay



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BROTH MACRODILUTION

MIC = 0.5 mg/l

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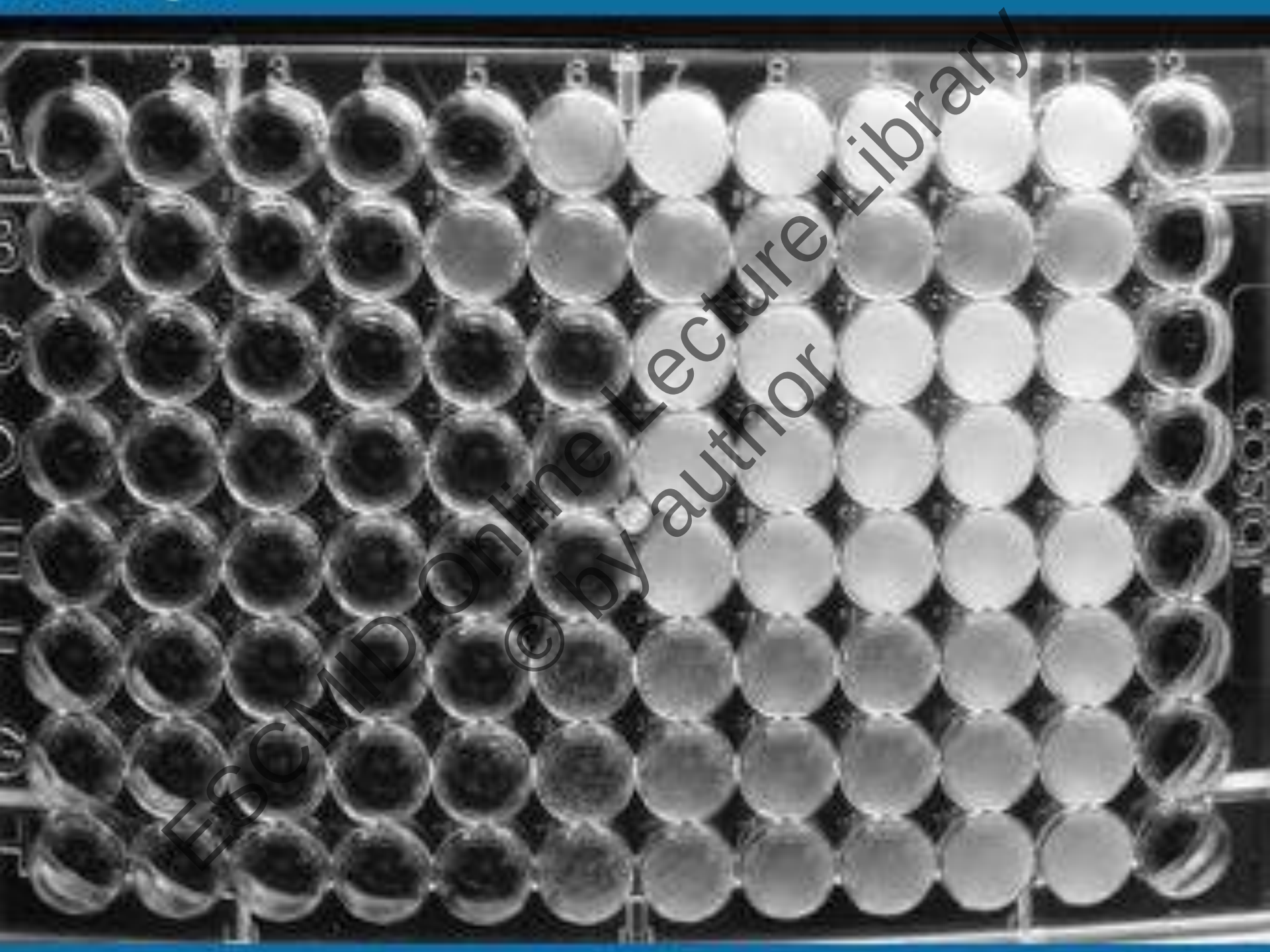
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Method for Reliable Determination of Minimal Lethal Antibiotic Concentrations

RICHARD D. PEARSON,[1]† ROY T. STEIGBIGEL,[1]* HENRY T. DAVIS,[2] AND STANLEY W. CHAPMAN[1]‡

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The concentration of the initial inoculum and the number and volume of samples subcultured are covariant parameters that affect the number of colonies in the sample(s) and, along with the definition of lethality, timing of subculture, and conditions of culture (i.e., temperature, atmosphere, growth phase, etc.), must be accounted for in a test procedure for MLCs.

Intrinsic sampling variability makes it impossible to use as a rejection value the number of colonies that represents precisely a 0.999 lethal effect. It was necessary to develop a test procedure that took this into account and also allowed for variability in pipetting. The Poisson distribution, as assessed by a chi-square goodness of fit test, is an acceptable probability model

MICRODILUTION

Advantages

- MIC and MBC determination
- Easy to perform
- Plates can be prepared in advance, and be conserved at –80°C
- Low antibiotic powder consumption
- Versatility
- Automation

Inconveniences

- Difficulties to detect contamination
- Intrinsic 1 dilution error

Reference Technique for most organisms

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AGAR DILUTION

ANTIMICROBIAL AGENT:

- Two-fold dilutions incorporated into the agar medium before it solidifies

INOCULUM:

-Dispensed with a multi-inoculator (Steers)

- 10^4 CFU/dot

MEDIUM:

- Similar to broth media PLUS ca. 15 g/L of agar

INTERPRETATION:

- Absence of growth on the surface of the plate (≤ 3-5 colonies-) (usual incubation: 18h-35ºC)

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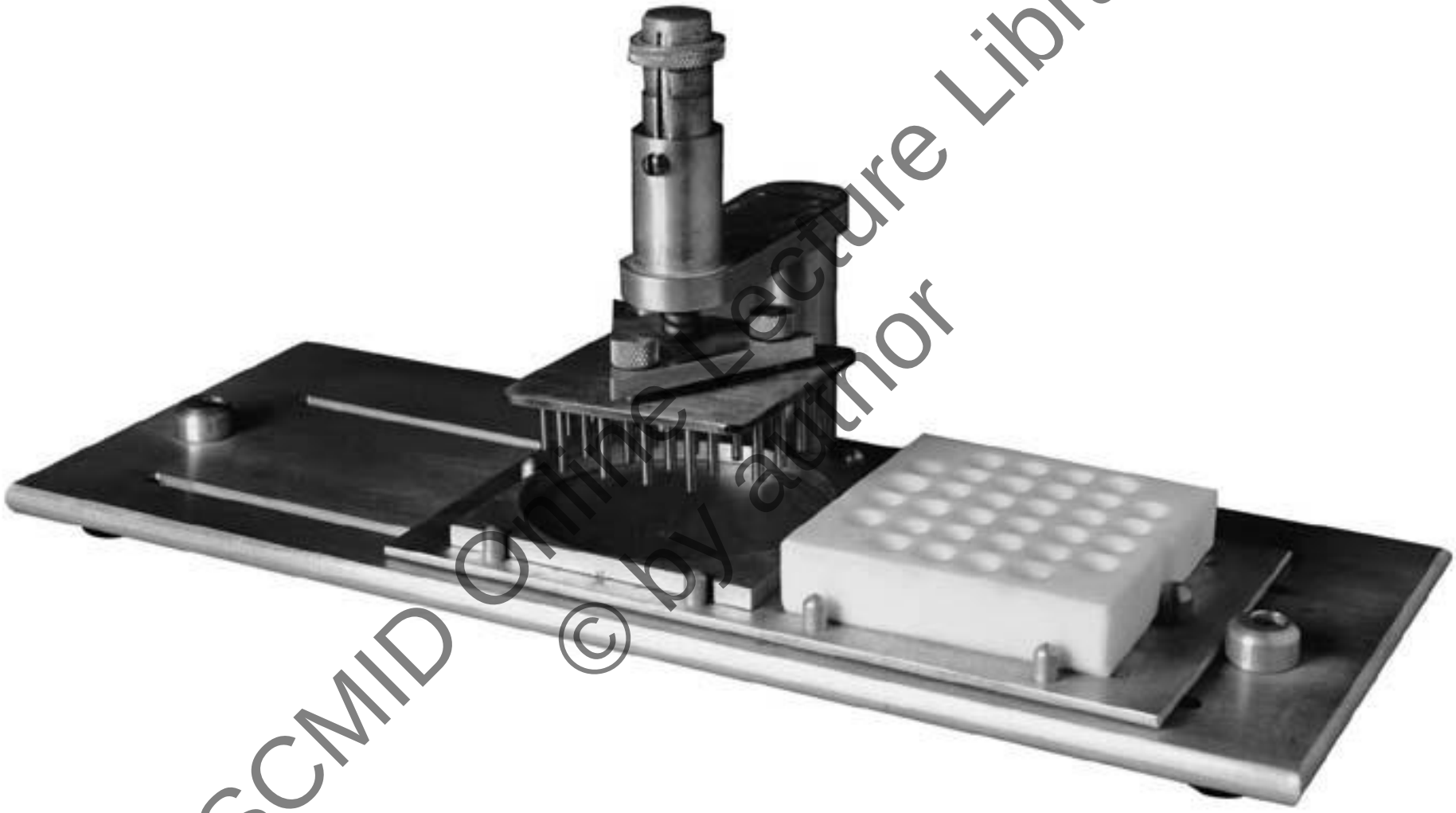
Steers replicator



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Steers, E., E. L. Foltz, and B. S. Graves. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. 9:307-311.

AGAR DILUTION

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AGAR DILUTION

Advantages

Simultaneous study of >= 32 microorganisms
Contaminations are easily detected

Inconveniences

CMB “cannot” be determined
A lot of antibiotic powder is consumed
Intrinsic 1 dilution error

CLSI Reference method for:

- ***Neisseria gonorrhoeae***
- ***Helicobacter pylori***

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OTHER SUSCEPTIBILITY ASSAYS

Population analysis

Post-antibiotic effect

Antimicrobial combinations

(checkerboard, killing curves)

Minimal antibiotic concentration

Mutant preventing concentration

Serum bactericidal (inhibitory) assay

[Antibiotic concentration in organic fluids]

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