



STATENS
SERUM
INSTITUT

Broth Microdilution

Robert Skov

Head of Bacteriological Surveillance and Infection Control

Statens Serum Institut

Copenhagen, Denmark

MIC standard

□ Microbroth dilution (ISO-standard)

➤ **ISO 20776-1 (2006)**

Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

- I.e. not developed for fastidious organisms!

INTERNATIONAL
STANDARD

ISO
20776-1

2006

Optical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices —
Part 1
Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases

ISO 20776-1:2006

Why a standard is needed

- ❑ MIC-values are not absolute – they vary with
 - inoculum,
 - incubation time,
 - substrate, including cations
 - Incubation atmosphere including pH,
 - The bigger the inoculum, the higher the MIC
 - The longer the incubation time the higher the MIC
 - methodology

Purpose of a standard

- ❑ Provides industry with a reference to evaluate new devices /technologies
 - **ISO 20776-2**
 - Provides a reference methodology for establishing an MIC



Broth

- Cation-supplemented Mueller Hinton broth containing an antibiotic in two-fold dilutions
 - Wells are filled with either 50 μ L antibiotic containing broth or 100 μ L antibiotic containing broth
 - May be prepared in advance and stored (sealed plastic bags at $<-60^{\circ}\text{C}$)

Inoculum

- Three to five colonies from a non-selective nutritive agar medium (incubated overnight)
 - touch with a loop and transferred to 0.9% saline.
 - The suspension is adjusted to give a turbidity equivalent to that of a 0.5 McFarland standard.
 - transfer into the wells within 30 minutes.

Inoculation

- Wells are inoculated with a bacterial suspension
 - final concentration of 5×10^5 CFU/well.
 - If the “50+ 50” is used - the 0.5 McFarland suspension is diluted x100 in 0.9% NaCl and 50 μ L is transferred
 - If the “100+5” is used - 5 μ L of the 0.5 McFarland standard is transferred.

Incubation

- ❑ Microdilution trays should be sealed in polyethylene bags or fitted with a tight lid or adhesive seal before incubation
 - to avoid uneven heating trays should not be stacked more than five high.
 - Trays are incubated overnight at 34-37°C in ambient air for 18 ± 2 h

Reading

- ❑ Results shall only be read when there is sufficient growth of the test organism
 - obvious button or definite turbidity in the positive growth control
 - no growth in the un-inoculated or negative growth control (where present)
 - and when purity and the appropriate cell number concentration of the inoculum has been established.
- ❑ The MIC is the lowest concentration that completely inhibits visible growth.

Organism	Culture collection numbers	Characteristics
<i>E. coli</i>	ATCC 25922; NCTC 12241; CIP 7624 DSM 1103; CCUG 17620	Susceptible, wild-type
<i>P. aeruginosa</i>	ATCC 27853; NCTC 12903; CIP 76110 DSM 1117; CCUG 17619	Susceptible, wild-type
<i>S. aureus</i>	ATCC 29213; NCTC 12973; CIP 103429 DSM 2569; CCUG 15915	Weak β -lactamase producer
<i>E. faecalis</i>	ATCC 29212; NCTC 12697; CIP 103214 DSM 2570; CCUG 9997	Susceptible, wild-type
<i>S. pneumoniae</i>	ATCC 49619; NCTC 12977; CIP 104340 DSM 11967; CCUG 33638	Penicillin intermediate
<i>H. influenzae</i>	NCTC 8468; CCUG 23946	Susceptible, wild-type

ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA.

NCTC, National Collection of Type Cultures, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5HT, UK.

CIP, Collection de Institut Pasteur, 25–28 Rue du Docteur Roux, 75724 Paris Cedex 15 France.

DSMZ, Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, Mascheroder Weg 16, D-38124 Braunschweig, Germany.

CCUG, The Culture Collection University of Gothenburg <http://www.ccug.se/>

MBD I rutinelab

- ❑ Har MBD en plads I almindelige laboratorier
 - Ja det mener jeg!
 - MIC bestemmelse er vigtigt ved visse alvorlige infektioner!
 - Gradient test er ikke altid det bedste alternativ
 - Proceduren er ikke vanskelig
 - Plader / bakker med antibiotika kan enten laves selv eller købes
 - Fordel at kunne lave dem selv
 - Antibiotika kan købes fx fra Sigma – ingen grund til at købe den dyreste variant!
 - » (alternativt kan man bruge infusionssubstans)



Table 1 — Examples of solvents and diluents for making stock solutions of selected antimicrobial agents¹⁾

Antimicrobial agent	Solvent	Diluent
Amikacin	Water	
Amoxicillin	Phosphate buffer 0,1 mol/l, pH 6,0	Phosphate buffer 0,1 mol/l, pH 6,0
Ampicillin	Phosphate buffer 0,1 mol/l, pH 8,0	Phosphate buffer 0,1 mol/l, pH 6,0
Azithromycin	Ethanol volume fraction 95 % or glacial acetic acid ^{a)}	Water
Azlocillin	Water	
Aztreonam	Saturated sodium bicarbonate solution	Water
Carbenicillin	Water	
Cefaclor	Water	
Cefamandole	Water	
Cefazolin	Phosphate buffer 0,1 mol/l, pH 6,0	Phosphate buffer 0,1 mol/l, pH 6,0
Cefdinir	Phosphate buffer 0,1 mol/l, pH 6,0	Water
Cefditoren	Phosphate buffer 0,1 mol/l, pH 6,0	Water
Cefepime	Phosphate buffer 0,1 mol/l, pH 6,0	Phosphate buffer 0,1 mol/l, pH 6,0
Cefetamet	Phosphate buffer 0,1 mol/l, pH 6,0	Water
Cefixime	Phosphate buffer 0,1 mol/l, pH 7,0	Phosphate buffer 0,1 mol/l, pH 7,0
Cefmetazole	Water	
Cefonicid	Water	
Cefoperazone	Water	
Cefotaxime	Water	
Cefotetan	Dimethyl sulfoxide	Water
Cefoxitin	Water	
Cefpodoxime	Mass concentration 0,1 % sodium bicarbonate solution	Water
Cefprozil	Water	
Ceftazidime	Saturated sodium bicarbonate solution	Water
Ceftibuten	1/10 volume of dimethyl sulfoxide	Water
Ceftizoxime	Water	
Ceftoliprole	Dimethyl sulfoxide plus glacial acetic acid ^{b)}	Water, vortex vigorously
Ceftriaxone	Water	
Cefuroxime	Phosphate buffer 0,1 mol/l, pH 6,0	Phosphate buffer 0,1 mol/l, pH 6,0
Cephalothin	Phosphate buffer 0,1 mol/l, pH 6,0	Water
Chloramphenicol	Ethanol volume fraction 95 %	Water
Cinoxacin	Half volume of water, a minimum volume 1 mol/l NaOH to dissolve, then make up to total volume with water	Water