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COMMITTEE REPORT

Ariaki Nagayama · Keizo Yamaguchi
Kunitomo Watanabe · Masatoshi Tanaka
Intetsu Kobayashi · Zenzo Nagasawa

Final report from the Committee on Antimicrobial Susceptibility Testing, Japanese Society of Chemotherapy, on the agar dilution method (2007)

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Abstract In 1968, the agar dilution method was developed as an independent Japanese method for measuring the minimal inhibitory concentration (MIC) of antimicrobial agents. As this method differed in a few respects from the MIC measurement methods used in other countries, it was revised in 1981, by a committee headed by Susumu Mitsuhashi, and the revised method (Chemotherapy 29:76–79, 1981) has been used since then.

In 1979, an agar dilution method for measuring the MIC of anaerobes was developed by a committee chaired by Nozomu Kosakai (Chemotherapy 27:559–561, 1979). In 1990, a committee headed by Sachiko Goto approved a broth microdilution method for nonfastidious bacteria (Chemotherapy 38:102–105, 1990). Later, a committee headed by Atsushi Saito examined media that would be suitable for nonfastidious bacteria and fastidious bacteria, and they endeavored to prepare a broth microdilution method for anaerobic bacteria. In this context, a new broth microdilution method was proposed at the 40th Annual Meeting of the Japanese Society of Chemotherapy (JSC) in Nagoya in 1992, and the proposal was adopted as the standard JSC method after some modification (Chemotherapy 41: 183–189, 1993).

The agar dilution method has remained unrevised for approximately 20 years. A proposal to review this method was recently made, and the 2007 Committee on Antimicrobial Susceptibility Testing was formed, comprising the JSC members listed below. Under the auspices of this committee, the method revised in 1981 was reviewed in comparison to the international standard method (Clinical and Laboratory Standards Institute [CLSI] method).

Key words Minimal inhibitory concentration (MIC) · Agar dilution method · Reference strain · Medium

Rationale for revision of the agar dilution method (standard JSC method)

In 1968, the agar dilution method was established as an independent Japanese method for measuring the minimal inhibitory concentration (MIC) of antimicrobial agents. The method was revised in 1981 by a committee headed by Susumu Mitsuhashi. This method has since been used for approximately 20 years without further modification. During this period, a method prepared by the Clinical and Laboratory Standards Institute (CLSI) has been widely accepted as the global standard. There are several striking differences between the Japanese standard method and the CLSI method of agar dilution, regarding the concentrations of the bacterial suspensions and the antibacterial agents used. Because these differences hamper the evaluation and comparison of data on an international scale, members of the JSC have advocated a revision of the current JSC method.

Committee on Antimicrobial Susceptibility Testing: mission statement for agar dilution method of measuring MIC

The Committee on Antimicrobial Susceptibility Testing (chaired by Ariaki Nagayama; hereafter, the Committee) held several discussion meetings and proposed adopting the CLSI method as the JSC standard method of agar dilution. This proposal was made because the CLSI method is based mostly on the information obtained by international cooperative studies, and it is practical enough to be adopted as a routine procedure at clinical microbiology laboratories in Japan. Furthermore, the CLSI method allows the evalua-

The Committee on Antimicrobial Susceptibility Testing, Japanese Society of Chemotherapy
(A. Nagayama, chairperson; K. Yamaguchi · K. Watanabe · M. Tanaka · I. Kobayashi · Z. Nagasawa, committee members.)

A. Nagayama (✉) · K. Yamaguchi · K. Watanabe · M. Tanaka · I. Kobayashi · Z. Nagasawa
The Committee on Antimicrobial Susceptibility Testing, Japanese Society of Chemotherapy, Nichinai Kaikan B1, 3-28-8 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
Tel. +81-3-5842-5533; Fax +81-3-5842-5133
e-mail: karyo@jc4.so-net.ne.jp

tion and comparison of data on an international scale and is compatible with the Japanese testing standard.

However, the Japanese method of agar dilution[1] needs to be referred to as a comparison when evaluating new data obtained with the CLSI method, because abundant data have been accumulated with the Japanese method.

The Japanese agar dilution method for anaerobes, which focuses on the genus *Bacteroides*, also requires some modifications and additions, including items such as the medium for susceptibility testing, method of preparing bacterial suspension, method of anaerobic culture, and incubation time.

Taking this background into account, the present Committee decided to adopt the CLSI method of agar dilution[2–5] as one of the standard JSC methods, based on evaluation and comparison of its performance with the JSC method when used for measuring the MIC of bacteria in general, and of anaerobes in particular.

CLSI agar dilution method for measuring MIC[1]

Organisms covered

The CLSI agar dilution method for measuring MIC[1] is applicable to the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, nonglucose-fermenting bacteria other than *Pseudomonas aeruginosa*, the genus *Acinetobacter*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Vibrio cholerae*, *Staphylococcus* spp., enterococci, *Neisseria gonorrhoeae*, streptococci other than pneumococci, *Neisseria meningitides*, *Helicobacter pylori*, and anaerobes.

Media for susceptibility testing

Organisms

The family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, nonglucose-fermenting bacteria other than *Pseudomonas aeruginosa*, the genus *Acinetobacter*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Vibrio cholerae*, *Staphylococcus* spp., and enterococci.

Mueller-Hinton agar (MHA; composition: meat extract [300 g], casamino acid [17.5 g], starch [1.5 g), agar [17 g], and purified water [1000 ml]) is used as the medium for susceptibility testing. To evaluate the activity of oxacillin against *Staphylococcus* spp., MHA supplemented with 2% (w/v) sodium chloride is used.

Neisseria gonorrhoeae

GC agar medium with 1% supplement (for stimulation of growth) is used.

The composition of the supplement (per l) is: L-cysteine (1.1 g), guanine hydrochloride (0.03 g), thiamine hydrochloride (3 mg), *p*-aminobenzoic acid [PABA] (13 mg), vitamin B12 (0.01 g), cocarboxylase (0.1 g), nicotinamide adenine dinucleotide [NAD] (0.25 g), adenine (1.0 g), L-glutamine (10 g), glucose (100 g), and iron (II) nitrate (0.02 g).

Streptococci other than pneumococci

MHA supplemented with sheep blood (5% v/v) is used. Lysed horse blood is used when sulfonamides are assessed.

Neisseria meningitides

MHA supplemented with sheep blood (5% v/v) is used.

Helicobacter pylori

MHA supplemented with sheep blood (5% v/v; collected more than 2 weeks previously) is used.

Anaerobes

Brucella agar medium containing hemin (5 mg/ml), vitamin K_1 (1 mg/ml), and lysed horse blood (5%) is used.

Inoculum preparation for dilution test; turbidity standard for inoculum preparation

According to the CLSI method, a bacterial suspension is prepared either by a proliferation technique or by a direct technique. For each organism, a suspension (10^8 CFU/ml) is prepared both by the proliferation method and by the direct colony suspension method or only by the latter method. If a 10^8 CFU/ml suspension is to be prepared, a broth for bacterial proliferation or sterile physiological saline is used by adjusting the opacity to 0.5 McFarland (equivalent to $1–2 \times 10^8$ CFU/ml if *Escherichia coli* ATCC 25922 is used) with a specific nephelometer or macroscopically. If macroscopic adjustment is needed, our Committee recommends the use of two standard opacity solutions (0.5 and 1 McFarland). When organisms assuming a mucoid form (*Pseudomonas aeruginosa*, *Klebsiella*, etc.) are used, it is difficult to prepare a suspension of 0.5 McFarland opacity. For these organisms, the relationship between opacity and bacterial concentration is determined in advance.

When a 3-mm inoculation pin is used, the suspension with 0.5 McFarland opacity is diluted 1:10 with sterile broth or physiological saline. If a 1-mm pin is used, the suspension with 0.5 McFarland opacity is used without dilution.

Proliferation technique

The family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, nonglucose-fermenting bacteria other than *Pseudomonas aeruginosa*, the genus *Acinetobacter*, *Burkholderia cepacia*,

Stenotrophomonas maltophilia, *Vibrio cholerae*, and enterococci.

Direct technique

The family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, nonglucose-fermenting bacteria other than *Pseudomonas aeruginosa*, the genus *Acinetobacter*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Vibrio cholerae*, *Staphylococcus* spp., *Enterococcus* spp., *Neisseria gonorrhoeae*, *Staphylococcus* spp other than *Staphylococcus pneumoniae*, *Neisseria meningitides,* and *Helicobacter pylori*.

Note that *Helicobacter pylori* should be passaged for 72 h in blood agar medium and made into a 2 McFarland suspension (1×10^7–10^8 CFU/ml).

The following methods are recommended for preparing suspensions of anaerobes:

(1) Bacterial proliferation technique
An adequate number of colonies are inoculated onto thioglycolate medium supplemented with hemin and menadione (containing no indicator), followed by incubation at 35°C–37°C until an appropriate opacity is recorded (for 6–24 h).

(2) Direct technique
Colonies incubated for 24–48 h in an agar medium appropriate for anaerobes are directly suspended in a liquid medium for bacterial suspension. This manipulation needs to be completed within 30 min after the anaerobic conditions are suspended.

(3) Method for adjusting suspension
Regardless of whether the proliferation technique or the direct technique is used, a suspension equivalent in opacity to 0.5 McFarland standard solution is finally prepared, as a rule (opacity needs to be adjusted depending on the organism; see the rules[6] attached to the *Report from the Bacterial Susceptibility Assessment Committee*, Japanese Society of Chemotherapy, 1993, Liquid media for bacterial suspensions should be those with a high transparency level (e.g., broth for anaerobes) which have been reduced in advance or degassed (boiled and cooled rapidly) before use. Each suspension is prepared immediately before inoculation.

Method for preparing antibacterial agents

With the CLSI method, antibacterial agents in bulk powder need to be obtained from reliable suppliers, accompanied by a certificate carrying the generic name, lot number, potency, and expiration date. Each drug is dissolved in an appropriate solvent for that drug. The solution is stored at 2°C–8°C, as a rule, because the potencies of some drugs decrease rapidly. Thus, it should be understood that the manipulations are to be carried out immediately after each suspension is made. Also, as a rule, the concentration of the antibacterial drug solution is set at the level of 1 μg/ml, and additional concentration levels are prepared by serial multiplications by certain factors (e.g., 128, 64, 32 μg/ml). However, depending on the purpose of the test, concentration levels may be set in different ways.

Preparing agar dilution plates

Media for susceptibility testing are kept warmed at 45°C–50°C. The antibacterial drug solution is combined with the medium at an appropriate ratio in a dish. The mixture is thoroughly agitated until it becomes solid. The agar thickness should be 3–4 mm. As a rule, the medium for anaerobes is prepared on the day of use and is not stored.

Inoculating agar dilution plates

With the CLSI method, the prepared/diluted bacterial suspension (10^7 CFU/ml) is inoculated onto the agar surface at a concentration of $1–3 \times 10^4$ CFU/spot, using a prescribed loop, pipette, or other device for inoculation. When a large number of test bacteria are inoculated at one time, a metallic pin (usually 3 mm in diameter) is used. As the devices used for inoculation are distributed by various companies, the amount of inoculation suitable for use with each metallic pin should be checked in advance. Anaerobes should be inoculated at a concentration of $1–3 \times 10^5$ CFU/spot onto the agar surface. Many anaerobes can endure brief exposure to ambient air, and so all such manipulations can be carried out under ambient air. Some bacteria or bacterial strains require all manipulations to be performed in an anaerobic glove box.

Care is needed regarding the following:

(1) For bacteria in general, inoculation should be performed on one drug-free plate. For anaerobes, inoculation should be performed on two plates (one for control of the anaerobic culture and the other for control of the aerobic culture) to check the growth of anaerobes and contamination by facultative bacteria at the beginning and end of bacterial suspension inoculation.
(2) The bacterial suspension is first inoculated onto the plate with the lowest drug concentration and then onto plates with higher drug concentrations.
(3) If the test drug is changed, inoculation onto a drug-free medium is performed to confirm the absence of any influence from the previously tested drug.

Incubating agar dilution plates

The CLSI method specifies the conditions and duration of culture for each organism.

Organisms

The family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, nonglucose-fermenting bacteria other than *Pseudomonas*

Table 1. Quality control (QC) ranges for antibacterial drugs

Drug	Criterion range	*Staphylococcus aureus* ATCC 29213 (μg/ml)	*Enterococcus faecalis* ATCC 29212 (μg/ml)	*Escherichia coli* ATCC 25922 (μg/ml)	*Pseudomonas aeruginosa* ATCC 27853 (μg/ml)	*Escherichia coli* ATCC 35218 (μg/ml)
Amikacin	CLSI	1–4	64–256	0.5–4	1–4	–
Amoxicillin	CLSI	–	–	–	–	–
Amoxicillin-clavulanic acid	CLSI	0.125/0.06–0.5/0.25	0.25/0.125–1/0.5	2/1–8/4	–	4/2–16/8
Ampicillin	CLSI	0.5–2	0.5–2	2–8	–	–
Ampicillin-sulbactam	CLSI	–	–	2/1–8/4	–	8/4–32/16
Arbekacin	JSC[a)]	0.25–1	16–64	0.5–2	1–4	1–4
Aspoxicillin	JSC[a)]	2–8	2–8	2–8	16–64	–
Astromicin	JSC[a)]	1–4	16–64	2–8	–	–
Azithromycin	CLSI	0.5–2	–	–	–	–
Azlocillin	CLSI	2–8	1–4	8–32	2–8	–
Aztreonam	CLSI	–	–	0.06–0.25	2–8	–
Biapenem	JSC[a)]	0.03–0.125	0.5–2	0.03–0.125	0.5–2	0.03–0.125
Carbenicillin	CLSI	2–8	16–64	4–16	16–64	–
Carumonam	JSC[a)]	–	–	0.06–0.5	1–4	–
Cefaclor	CLSI	1–4	–	1–4	–	–
Cefamandole	CLSI	0.25–1	–	0.25–1	–	–
Cefatrizine	JSC[a)]	0.5–2	–	4–16	–	–
Cefazolin	CLSI	0.25–1	–	1–4	–	–
Cefbuperazone	JSC[a)]	8–32	–	0.125–1	–	–
Cefdinir	CLSI	0.125–0.5	–	0.125–0.5	–	–
Cefditoren	CLSI	0.25–2	–	0.125–1	–	–
Cefepime	CLSI	1–4	–	0.015–0.125	1–8	–
Cefetamet	CLSI	–	–	0.25–1	–	–
Cefixime	CLSI	8–32	–	0.25–1	–	–
Cefmenoxime	JSC[a)]	1–4	–	0.06–0.5	8–32	–
Cefmetazole	CLSI	0.5–2	–	0.25–1	>32	–
Cefminox	JSC[a)]	8–32	–	0.25–1	–	–
Cefodizime	JSC[a)]	4–16	–	–	16–64	–
Cefonicid	CLSI	1–4	–	0.25–1	–	–
Cefoperazone	CLSI	1–4	–	0.125–0.5	2–8	–
Cefoselis	JSC[a)]	0.5–2	–	0.03–0.125	1–4	–
Cefotaxime	CLSI	1–4	–	0.03–0.125	8–32	–
Cefotetan	CLSI	4–16	–	0.06–0.25	–	–
Cefotiam	JSC[a)]	0.5–2	–	0.125–0.5	–	0.06–0.25
Cefoxitin	CLSI	1–4	–	2–8	–	–
Cefozopran	JSC[a)]	0.5–2	–	0.06–0.25	0.5–2	–
Cefpiramide	JSC[a)]	1–4	–	1–4	1–4	2–8
Cefpirome	JSC[a)]	0.25–1	1–8	0.03–0.125	1–4	<0.5
Cefpodoxime	CLSI	1–8	–	0.25–1	–	–
Cefprozil	CLSI	0.25–1	–	1–4	–	–
Cefroxadine	JSC[a)]	1–4	–	2–8	–	–
Cefsulodin	JSC[a)]	2–8	–	32–128	1–4	32–128
Ceftaroline	CLSI	0.125–0.5	–	0.03–0.125	–	–
Ceftazidime	CLSI	4–16	–	0.06–0.5	1–4	–
Cefteram	JSC[a)]	2–8	>1	0.125–0.5	32–128	–
Ceftibuten	CLSI	–	–	0.125–0.5	–	–
Ceftizoxime	CLSI	2–8	–	0.03–0.125	16–64	–
Ceftobiprole	CLSI	0.25–1	0.06–0.5	0.03–0.125	1–4	–
Ceftriaxone	CLSI	1–8	–	0.03–0.125	8–64	–
Cefuroxime	CLSI	0.5–2	–	2–8	–	–
Cephalexin	JSC[a)]	1–4	–	8–32	–	–
Cephalothin	CLSI	0.125–0.5	–	4–16	–	–
Chloramphenicol	CLSI	2–16	4–16	2–8	–	–
Cinoxacin	CLSI	–	–	2–8	–	–
Ciprofloxacin	CLSI	0.125–0.5	0.25–2	0.004–0.015	0.25–1	–
Clarithromycin	CLSI	0.125–0.5	–	–	–	–
Clinafloxacin	CLSI	0.008–0.06	0.03–0.25	0.002–0.015	0.06–0.5	–
Clindamycin	CLSI	0.06–0.25	4–16	–	–	–
Cloxacillin	JSC[a)]	0.25–1	8–32	256–1024	–	–
Colistin	CLSI	–	–	0.25–1	0.25–2	–
Dalbavancin	CLSI	0.03–0.125	0.03–0.125	–	–	–
Daptomycin	CLSI	0.25–1	1–4	–	–	–
Dibekacin	JSC[a)]	0.25–2	8–32	0.5–4	0.5–4	–
Dicloxacillin	JSC[a)]	0.125–0.5	8–32	–	–	–
Dirithromycin	CLSI	1–4	–	–	–	–
Doripenem	CLSI	0.015–0.06	1–4	0.015–0.06	0.125–0.5	–
Doxycycline	CLSI	0.125–0.5	2–8	0.5–2	–	–

Streptococcus pneumoniae ATCC 49619 (μg/ml)	*Neisseria gonorrhoeae* ATCC 49226 (μg/ml)	*Helicobacter pylori* ATCC 43504 (μg/ml)	*Bacteroides fragilis* ATCC 25285 (μg/ml)	*Bacteroides thetaiotaomicron* ATCC 29741 (μg/ml)	*Clostridium difficile* ATCC 700057 (μg/ml)	*Eggerthella lenta* ATCC 43055 (μg/ml)
–	–	–	–	–	–	–
0.03–0.12	–	0.015–0.125	–	–	–	–
0.03/0.015–0.12/0.06	–	–	0.25/0.125–1/0.5	0.5/0.25–2/1	0.25/0.125–1/0.5	–
0.06–0.25	–	–	16–64	16–64	1–4	–
–	–	–	0.5/0.25–2/1	0.5/0.25–2/1	0.5/0.25–4/2	0.25/0.125–2/1
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.06–0.25	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
1–4	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.03–0.25	0.008–0.03	–	–	–	–	–
0.015–0.12	–	–	–	–	–	–
0.03–0.25	0.015–0.06	–	–	–	–	–
0.5–2	0.015–0.25	–	–	–	–	–
–	0.004–0.03	–	–	–	–	–
–	–	–	–	–	–	–
–	0.5–2	–	8–32	32–128	–	4–16
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	32–128	32–128	–	32–128
–	–	–	–	–	–	–
0.03–0.12	0.015–0.06	–	8–32	16–64	–	64–256
–	0.5–2	–	4–16	32–128	–	32–128
–	–	–	–	–	–	–
–	0.5–2	–	4–16	8–32	–	4–16
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.03–0.12	0.03–0.125	–	–	–	–	–
0.25–1	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.008–0.03	–	–	–	–	–	–
–	0.03–0.125	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.12–0.5	0.008–0.03	–	–	4–16	–	16–64
0.004–0.03	–	–	–	–	–	–
0.03–0.12	0.004–0.015	–	32–128	64–256	–	–
0.25–1	0.25–1	–	–	–	–	–
–	–	–	–	–	–	–
0.5–2	–	–	–	–	–	–
2–8	–	–	2–8	4–16	–	–
–	–	–	–	–	–	–
–	0.001–0.008	–	–	–	–	–
0.03–0.12	–	0.015–0.125	–	–	–	–
0.03–0.12	–	–	0.03–0.125	0.06–0.5	–	0.03–0.125
0.03–0.12	–	–	0.5–2	2–8	2–8	0.06–0.25
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.008–0.03	–	–	–	–	–	–
0.06–0.5	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.06–0.25	–	–	–	–	–	–
0.03–0.12	–	–	–	–	0.5–4	–
0.015–0.12	–	–	–	–	–	–

Table 1. *Continued*

Drug	Criterion range	*Staphylococcus aureus* ATCC 29213 (μg/ml)	*Enterococcus faecalis* ATCC 29212 (μg/ml)	*Escherichia coli* ATCC 25922 (μg/ml)	*Pseudomonas aeruginosa* ATCC 27853 (μg/ml)	*Escherichia coli* ATCC 35218 (μg/ml)
Enoxacin	CLSI	0.5–2	2–16	0.06–0.25	2–8	–
Ertapenem	CLSI	0.06–0.25	4–16	0.04–0.015	2–8	–
Erythromycin	CLSI	0.25–1	1–4	–	–	–
Faropenem	CLSI	0.03–0.125	–	0.25–1	–	–
Fleroxacin	CLSI	0.25–1	2–8	0.03–0.125	1–4	–
Flomoxef	JSC[a)]	0.25–1	32–128	0.06–0.25	–	–
Fosfomycin	CLSI	0.5–4	32–128	0.5–2	2–8	–
Garenoxacin	CLSI	0.004–0.03	0.03–0.25	0.004–0.03	0.5–2	–
Gatifloxacin	CLSI	0.03–0.125	0.125–1.0	0.008–0.03	0.5–2	–
Gemifloxacin	CLSI	0.008–0.03	0.015–0.125	0.004–0.015	0.25–1	–
Gentamicin	CLSI	0.125–1	4–16	0.25–1	0.5–2	–
Grepafloxacin	CLSI	0.03–0.125	0.125–0.5	0.004–0.03	0.25–2.0	–
Iclaprim	CLSI	0.06–0.25	0.004–0.03	1–4	–	–
Imipenem	CLSI	0.015–0.06	0.5–2	0.06–0.25	1–4	–
Isepamicin	JSC[a)]	1–4	32–128	0.5–2	2–8	0.5–2
Josamycin	JSC[a)]	1–4	2–8	–	–	–
Kanamycin	CLSI	1–4	16–64	1–4	–	–
Kitasamycin	JSC[a)]	0.25–1	1–4	–	–	–
Latamoxef	JSC[a)]	4–16	–	0.125–0.5	8–32	–
Levofloxacin	CLSI	0.06–0.5	0.25–2	0.008–0.06	0.5–4	–
Lincomycin	JSC[a)]	0.25–1	32–128	–	–	–
Linezolid	CLSI	1–4	1–4	–	–	–
Lomefloxacin	CLSI	0.25–2	2–8	0.03–0.125	1–4	–
Loracarbef	CLSI	0.5–2	–	0.5–2	>8	–
Mecillinam	CLSI	–	–	0.03–0.25	–	–
Meropenem	CLSI	0.03–0.125	2–8	0.008–0.06	0.25–1	–
Methicillin	CLSI	0.5–2	>16	–	–	–
Metronidazole	CLSI	–	–	–	–	–
Mezlocillin	CLSI	1–4	1–4	2–8	8–32	–
Micronomicin	JSC[a)]	0.125–1	4–32	0.5–2	2–8	–
Minocycline	CLSI	0.06–0.5	1–4	0.25–1	–	–
Moxalactam	CLSI	4–16	–	0.125–0.5	8–32	–
Moxifloxacin	CLSI	0.015–0.125	0.06–0.5	0.008–0.06	1–8	–
Mupirocin	JSC[a)]	0.125–0.5	32–128	32–128	–	–
Nafcillin	CLSI	0.125–0.5	2–8	–	–	–
Nalidixic acid	CLSI	–	–	1–4	–	–
Netilmicin	CLSI	≦0.25	4–16	≦0.5–1	0.5–8	–
Nitazoxanide	CLSI	–	–	–	–	–
Nitrofurantoin	CLSI	8–32	4–16	4–16	–	–
Norfloxacin	CLSI	0.5–2	2–8	0.03–0.125	1–4	–
Ofloxacin	CLSI	0.125–1	1–4	0.015–0.125	1–8	–
Oritavancin	CLSI	0.5–2	0.125–1	–	–	–
Oxacillin	CLSI	0.125–0.5	8–32	–	–	–
Panipenem	JSC[a)]	0.03–0.125	0.25–1	0.03–0.125	4–16	–
Pazufloxacin	JSC[a)]	0.125–0.5	2–8	0.015–0.06	0.5–2	–
Penicillin	CLSI	0.25–2	1–4	–	–	–
Pipemidic acid	JSC[a)]	32–128	>32	1–4	16–64	–
Piperacillin	CLSI	1–4	1–4	1–4	1–8	–
Piperacillin-tazobactam	CLSI	0.25/4–2/4	1/4–4/4	1/4–4/4	1/4–8/4	0.5/4–2/4
Polymyxin B	CLSI	–	–	0.25–2	0.25–2	–
Prulifloxacin	JSC[a)]	0.125–0.5	0.25–2	0.008–0.06	0.125–1	–
Quinupristin-dalfopristin	CLSI	0.25–1	2–8	–	–	–
Ramoplanin	CLSI	–	–	–	–	–
Rifampin	CLSI	0.004–0.015	0.5–4	4–16	16–64	–
Rifaximin	CLSI	–	–	–	–	–
Rokitamycin	JSC[a)]	0.25–1	1–4	–	–	–
Roxithromycin	JSC[a)]	0.5–2	2–8	–	–	–
Sisomicin	JSC[a)]	0.125–1	8–64	0.25–2	0.5–4	–
Sparfloxacin	CLSI	0.03–0.125	0.125–0.5	0.004–0.015	0.5–2	–
Spectinomycin	CLSI	–	–	–	–	–
Sulbenicillin	JSC[a)]	2–8	16–64	8–32	16–64	–
Sulfamethoxazole-trimethoprim	CLSI	≦9.5/0.5	≦9.5/0.5	≦9.5/0.5	152/8–608/32	–
Sulfisoxazole	CLSI	32–128	32–128	8–32	–	–
Sultamicillin	JSC[a)]	0.25–1	1–4	4–16	–	8–32
Teicoplanin	CLSI	0.25–1	0.06–0.25	–	–	–
Telavancin	CLSI	0.125–1	0.125–0.5	–	–	–
Telithromycin	CLSI	0.06–0.25	0.015–0.125	–	–	–

Streptococcus pneumoniae ATCC 49619 (μg/ml)	*Neisseria gonorrhoeae* ATCC 49226 (μg/ml)	*Helicobacter pylori* ATCC 43504 (μg/ml)	*Bacteroides fragilis* ATCC 25285 (μg/ml)	*Bacteroides thetaiotaomicron* ATCC 29741 (μg/ml)	*Clostridium difficile* ATCC 700057 (μg/ml)	*Eggerthella lenta* ATCC 43055 (μg/ml)
–	0.015–0.06	–	–	–	–	–
0.03–0.25	–	–	0.06–0.25	0.25–1	–	0.5–2
0.03–0.12	–	–	–	–	–	–
0.03–0.25	–	–	0.03–0.25	0.125–1	–	1–4
–	0.008–0.03	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.015–0.06	–	–	0.06–0.5	0.25–1	0.5–2	1–4
0.12–0.5	0.002–0.015	–	–	–	–	–
0.008–0.03	–	–	–	–	–	–
–	–	–	–	–	–	–
0.06–0.5	0.004–0.03	–	–	–	–	–
0.03–0.12	–	–	–	–	–	–
0.03–0.12	–	–	0.03–0.125	0.125–0.5	–	0.125–0.5
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.5–2	–	–	–	–	–	–
–	–	–	–	–	–	–
0.5–2	–	–	2–8	2–8	1–4	0.5–2
–	0.008–0.03	–	–	–	–	–
2–8	–	–	–	–	–	–
–	–	–	–	–	–	–
0.06–0.25	–	–	0.03–0.25	0.125–0.5	0.5–4	0.125–1
–	–	–	–	–	–	–
–	–	64–256	0.25–1	0.5–2	0.125–0.5	–
–	–	–	16–64	8–32	–	8–32
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
0.06–0.25	–	–	0.125–0.5	1–4	1–4	0.125–0.5
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	0.06–0.5	–
4–16	–	–	–	–	–	–
–	–	–	–	–	–	–
–	0.004–0.015	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	0.25–1	–	8–32	8–32	1–4	–
–	–	–	–	–	–	–
–	–	–	2–8	8–32	4–16	8–32
–	–	–	0.125/4–0.5/4	4/4–16/4	4/4–16/4	4/4–16/4
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	0.125–0.5	–
–	–	–	–	–	–	–
–	–	–	–	–	0.0039–0.0156	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	0.004–0.015	–	–	–	–	–
–	8–32	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	0.06–0.5	–	–	–	–

Table 1. *Continued*

Drug	Criterion range	*Staphylococcus aureus* ATCC 29213 (μg/ml)	*Enterococcus faecalis* ATCC 29212 (μg/ml)	*Escherichia coli* ATCC 25922 (μg/ml)	*Pseudomonas aeruginosa* ATCC 27853 (μg/ml)	*Escherichia coli* ATCC 35218 (μg/ml)
Tetracycline	CLSI	0.125–1	8–32	0.5–2	8–32	–
Ticarcillin	CLSI	2–8	16–64	4–16	8–32	–
Ticarcillin-clavulanic acid	CLSI	0.5/2–2/2	16/2–64/2	4/2–16/2	8/2–32/2	8/2–32/2
Tigecycline	CLSI	0.03–0.25	0.03–0.125	0.03–0.25	–	–
Tinidazole	CLSI	–	–	–	–	–
Tizoxanide	CLSI	–	–	–	–	–
Tobramycin	CLSI	0.125–1	8–32	0.25–1	0.25–1	–
Tosufloxacin	JSC[a)]	0.03–0.125	0.125–0.5	–	0.125–0.5	–
Trimethoprim	CLSI	1–4	0.125–0.5	0.5–2	>64	–
Trimethoprim-sulfamethoxazole	CLSI	≦0.5/9.5	≦0.5/9.5	≦0.5/9.5	8/152–32/608	–
Trospectomycin	CLSI	2–16	2–8	8–32	–	–
Trovafloxacin	CLSI	0.008–0.03	0.06–0.25	0.004–0.015	0.25–2	–
Vancomycin	CLSI	0.5–2	1–4	–	–	–

[a] Criterion range tentatively set by the Japanese Society of Chemotherapy (to be used only as reference information)
[b] Precautions and other details are given in the CLSI *Performance standards for antimicrobial susceptibility testing* (latest edition)[4]

aeruginosa, the genus *Acinetobacter*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Vibrio cholerae*, *Staphylococcus* spp., and *Enterococcus* spp.

Culture

Incubation is done under aerobic conditions at 35 ± 2°C for 16–20 h (conditions can vary depending on the organisms and drugs).

(1) *Yersinia pestis* is incubated for 24 h, and the genus *Acinetobacter*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* are incubated for 20–24 h.
(2) Incubation is continued for 24 h, if the susceptibility of *Staphylococcus* spp. to oxacillin and vancomycin is being assessed. The temperature during incubation may not exceed 35°C when oxacillin-resistant *Staphylococcus* spp. are to be detected.
(3) Incubation is continued for 24 h if the sensitivity of enterococci to vancomycin is being assessed.

Neisseria gonorrhoeae

Culture

Incubation is done under 5% CO_2 at 36 ± 1°C for 20–24 h (temperature may not exceed 37°C).

Staphylococcus spp other than *Staphylococcus pneumoniae*

Culture

Incubation is done under aerobic conditions at 35 ± 2°C for 20–24 h (conditions vary depending on the organisms and drugs). Note the following:

(1) Incubation in the presence of CO_2 is permitted, if necessary for growth.
(2) If CO_2 is used, it should be kept in mind that the MIC may be affected by CO_2 if the test drug is stable in the alkaline pH range.

Neisseria meningitides

Culture

Incubated under 5% CO_2 at 35 ± 2°C for 20–24 h.

Helicobacter pylori

Culture

Incubated under slightly aerobic conditions at 35 ± 2°C for 72 h.

Incubation should be carried out under slightly aerobic conditions created by a gas generator to which bacteria of the genus *Campylobacter* are adaptable.

Anaerobic bacteria

Culture

Incubation is done under anaerobic conditions at 35–37°C for 42–48 h. Note the following:

(1) The control medium used to check for contamination by facultative bacteria is incubated under 5% CO_2.
(2) An anaerobic glove box or a container for anaerobic culture is used.
(3) The CLSI method specifies that the CO_2 concentration be 4%–7% (corresponding to the use of the Gas Pack method).
(4) The CO_2 level should be set at approximately 10%, or even less, although bacterial growth is stimulated at higher CO_2 levels.

Streptococcus pneumoniae ATCC 49619 (μg/ml)	*Neisseria gonorrhoeae* ATCC 49226 (μg/ml)	*Helicobacter pylori* ATCC 43504 (μg/ml)	*Bacteroides fragilis* ATCC 25285 (μg/ml)	*Bacteroides thetaiotaomicron* ATCC 29741 (μg/ml)	*Clostridium difficile* ATCC 700057 (μg/ml)	*Eggerthella lenta* ATCC 43055 (μg/ml)
–	0.25–1	0.125–1.0	0.125–0.5	8–32	–	–
–	–	–	16–64	16–64	16–64	16–64
–	–	–	–	0.5/2–2/2	16/2–64/2	16/2–64/2
–	–	–	0.125–1	0.5–2	0.125–1	0.06–0.5
–	–	–	–	–	0.125–0.5	–
–	–	–	–	–	0.06–0.5	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	–	–	–	–	–	–
–	1–4	–	–	–	–	–
–	0.004–0.015	–	–	–	–	–
–	–	–	–	–	0.5–4	–

(5) When reporting the test results, the CO_2 concentration used for culture should be documented.

Determining agar dilution end points

The concentration (terminal point) is adopted as the MIC when cultures are observed macroscopically under adequate illumination, referring to the following criteria:

(1) A concentration at which bacterial growth is markedly suppressed and no evident growth is evident.
(2) A concentration at which bacterial growth decreases gradually, and only slight growth or haze (traces of the inoculated bacterial suspension seen as a group of very small spots) is visible.
(3) A concentration at which bacterial growth is markedly suppressed but numerous small colonies persist.
(4) A concentration at which bacterial growth is markedly suppressed but several colonies of normal size persist.
(5) A concentration at which bacterial growth is markedly suppressed, but haze can still be seen.
(6) A concentration at which bacterial growth decreases gradually and a small number of large colonies are visible.
(7) When trimethoprim or any drug of the sulfonamide family is tested, an 80% or more decrease in bacterial colonies as compared with the control medium is rated as "negative."

Reference strains for quality control

Organisms

Family *Enterobacteriaceae*
Reference strains for quality control: Escherichia coli ATCC 25922; *Escherichia coli* ATCC 35218 is additionally used for the assessment of susceptibility to combined drug preparations containing beta-lactamase inhibitors.
Pseudomonas aeruginosa, nonglucose-fermenting bacteria other than Pseudomonas aeruginosa, the genus *Acinetobacter*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia.*
Reference strains for quality control: Pseudomonas aeruginosa ATCC 27853 and *Escherichia coli* ATCC 25922. *Escherichia coli* ATCC 35218 is additionally used for the assessment of susceptibility to combined drug preparations containing beta-lactamase inhibitors.

Vibrio cholerae
Reference strain for quality control: Escherichia coli ATCC 25922.

Staphylococcus spp.
Reference strains for quality control: Staphylococcus aureus ATCC 29213; *Escherichia coli* ATCC 35218 is additionally used for the assessment of susceptibility to combined drug preparations containing beta-lactamase inhibitors.

Enterococcus spp.
Reference strain for quality control: Enterococcus faecalis ATCC 29212.

Neisseria gonorrhoeae
Reference strain for quality control: Neisseria gonorrhoeae ATCC 49226.

Staphylococcus spp other than *Staphylococcus pneumoniae*
Reference strain for quality control: Streptococcus pneumoniae ATCC 49619.

Neisseria meningitides
Reference strains for quality control: Streptococcus pneumoniae ATCC 49619; *Escherichia coli* ATCC 25922 is used

for assessment of susceptibilities to ciprofloxacin, nalidixic acid, and minocycline.

Helicobacter pylori
Reference strain for quality control: Helicobacter pylori ATCC 43504.

Anaerobic bacteria

Reference strains for quality control: Bacteroides fragilis ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Eggerthella lenta* ATCC 43055, and *Clostridium difficile* ATCC 700057.

Quality control (QC) range for antibacterial drugs

As a rule, the QC range contained in the CLSI *Performance standards for antimicrobial susceptibility testing* (latest edition)[4] is used for the agar dilution method. For those drugs not listed in the CLSI standards, reference QC ranges are shown, together with the CLSI QC range, in Table 1. The reference QC ranges can be used solely as reference information for the assessment of susceptibilities to antibacterial agents.

When the CLSI QC range is applied, a bias within ± one tube range may be deemed as acceptable, but a bias exceeding two tubes requires re-measurement.

The QC range needs to be updated according to the latest version of the CLSI standards. Reference QC ranges may be subject to change (addition or modification) in the future.

Other items

(1) Measurement of the MIC using the agar dilution method should be performed as appropriate, referring to the latest version of the CLSI standards.

(2) When the agar dilution method is applied to the following bacteria and other organisms, not covered by the CLSI method, it is advisable to use liquid dilution, as described in other CLSI standards (see references): *Haemophilus influenzae, Haemophilus parainfluenzae, Streptococcus pneumoniae, Bacillus anthracis, Yersinia pestis, Burkholderia mallei, Burkholderia pseudomallei, Francisella tularensis, Brucella* spp., *Abiotrophia* spp., *Granulicatella* spp., *Aeromonas hydrophila Complex, Plesiomonas shigelloides, Bacillus* spp. (other than *B. anthracis*), *Campylobacter jejuni/coli, Corynebacterium* spp., *Erysipelothrix rhusiopathiae,* HACEK group (see below), *Lactobacillus* spp., *Leuconostoc* spp., *Listeria monocytogenes, Moraxella catarrhalis, Pasteurella spp., Pediococcus* spp., *Vibrio* spp. (other than *V. cholerae*). The HACEK group includes the genus *Haemophilus* (*H. aphrophilus, H. paraphrophilus, H. segnis*), *Actinobacillus actinomycetemcomitans, Cardiobacterium* spp., *Eikenella corrodens*, and *Kingella* spp.

(3) Bacterial strains for precision control are available (not free of charge) at Kanto Chemical (Tokyo, Japan).

References

1. Japanese Society of Chemotherapy. Second revision of the measuring method for MIC. Chemotherapy 1981;29:76–9.
2. Clinical and Laboratory Standards Institute. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; proposed guideline M45-P. Wayne, PA: CLSI; 2005.
3. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, seventh edition. M7-A7 Wayne, PA: CLSI; 2006.
4. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. M100 – S17. Wayne, PA: CLSI; 2007.
5. Clinical and Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, seventh edition. M11-A7. Wayne, PA: CLSI; 2007.
6. Saito A, Inamatsu T, Okada J, Oguri T, Kanno H, Kumon H, et al. Report from the committee on antimicrobial susceptibility testing, Japanese Society of Chemotherapy (1992). Chemotherapy 1993;41:183–9.