Issues in Antimicrobial Susceptibility Test and Appropriate Test Methods

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Purpose of Antimicrobial Susceptibility Test

- Obtain *in vitro* antimicrobial results for proper treatment
- Detect resistant isolates
- Create an antibiogram

Methods of Antimicrobial Susceptibility Test

- Agar Dilution
- Disk Diffusion
- Broth Microdilution
- E-test

Agar Dilution

＜Features＞

Inoculate agar plates supplemented with different antimicrobial concentrations.

Read the MIC based on the presence or absence of the growth.

＜Advantages＞

Standard method.

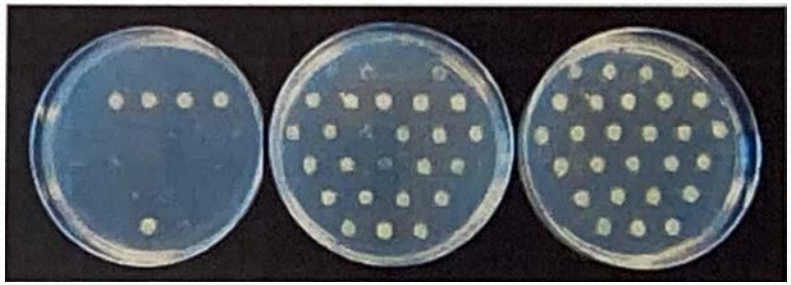
Quantitative results can be obtained with this method.

＜Disadvantages＞

Due to complicated preparation of agar plates and inoculation, the method is not suitable for routine test. Plates are prepared in-house.

Drug concentration（μg/mL）

2　　1　　0.5



Disk Diffusion

＜Features＞

Place drug-containing disks on an agar plate inoculated with targeted isolate. Measure the inhibition zones and determine S/R.

＜Advantages＞

Simple and affordable. Flexibility to choose which antimicrobial agents to test.

Possible to detect resistant isolates.

＜Disadvantages＞

Only S/R results can be obtained with this method. Inhibition zones are difficult to read in some organisms.

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Broth Microdilution

＜Features＞

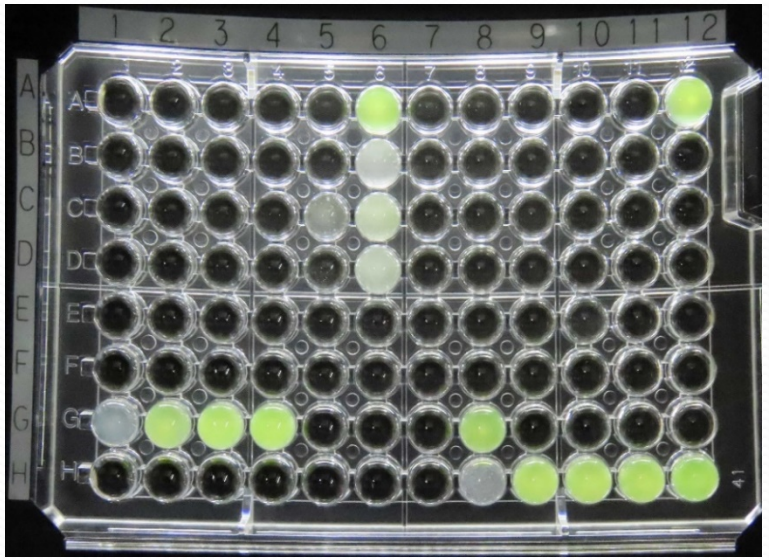
Inoculate dried or frozen panels containing different antimicrobial concentrations. Read the MIC based on the presence or absence of the growth.

＜Advantages＞

Measure MIC quantitatively.
Flexibility to choose which antimicrobial agent and concentrations to test. Tests can also be performed by automatic devices.

＜Disadvantages＞

Basically 1 plate/1 isolate.
Expensive compared to disk diffusion.



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E-Test

＜Features＞

Place drug-containing strips on an agar plate inoculated with targeted isolate and measure the MIC.

＜Advantages＞

Obtain MIC results easily.

Flexibility to choose which antimicrobial agent to test.

＜Disadvantages＞

Complicated if test multiple drugs at a time.

Difficult to read in some organisms.

※ Cited from Attached Document

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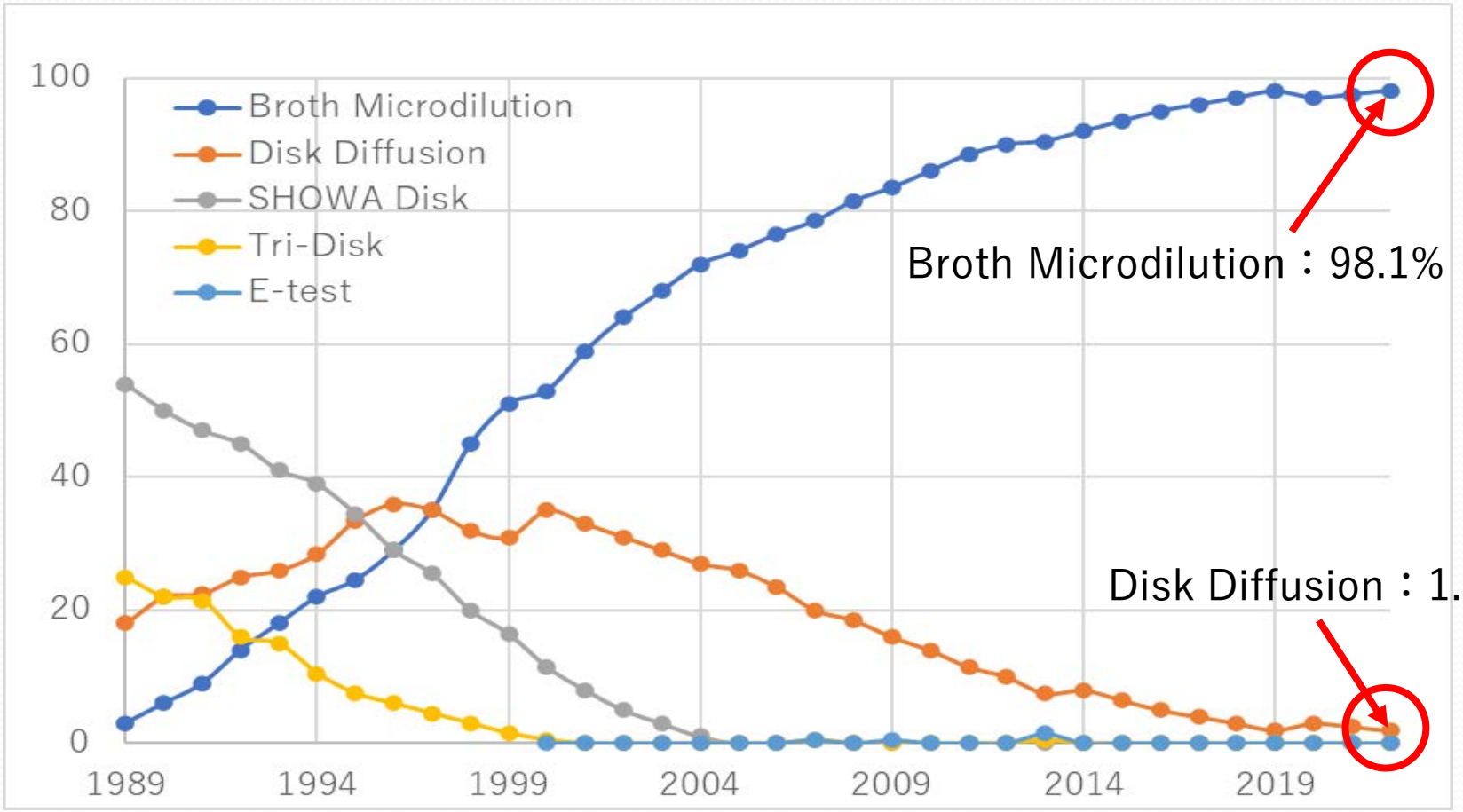
Changes in Antimicrobial susceptibility test



※ Cited from JAM Clinical test quality control survey report（2022）

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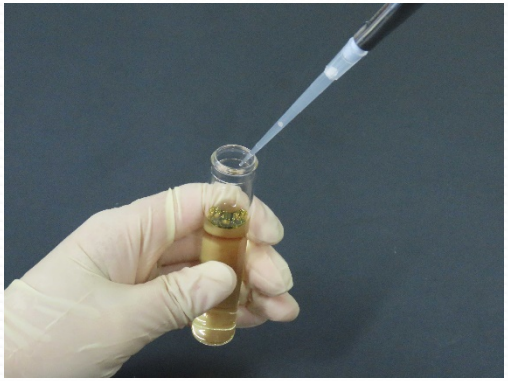
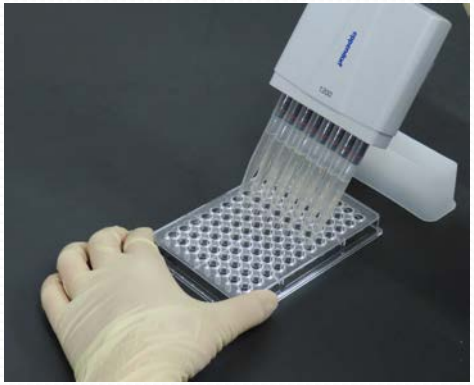
Procedure of Broth Microdilution

Targeted organism isolation

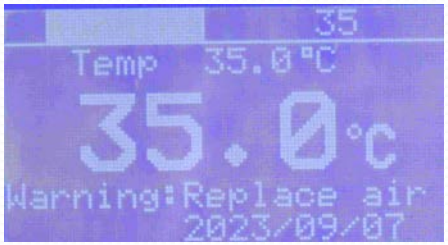
Inoculum preparation



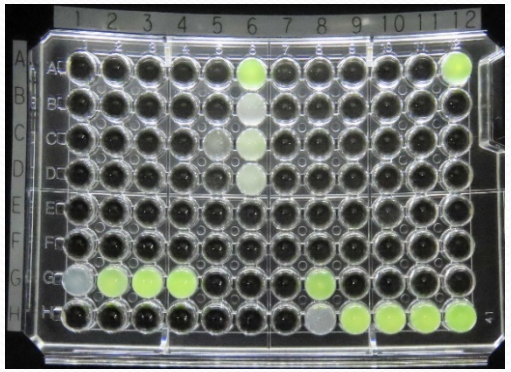
Inoculum transfer



Inoculation



Incubation



Reading

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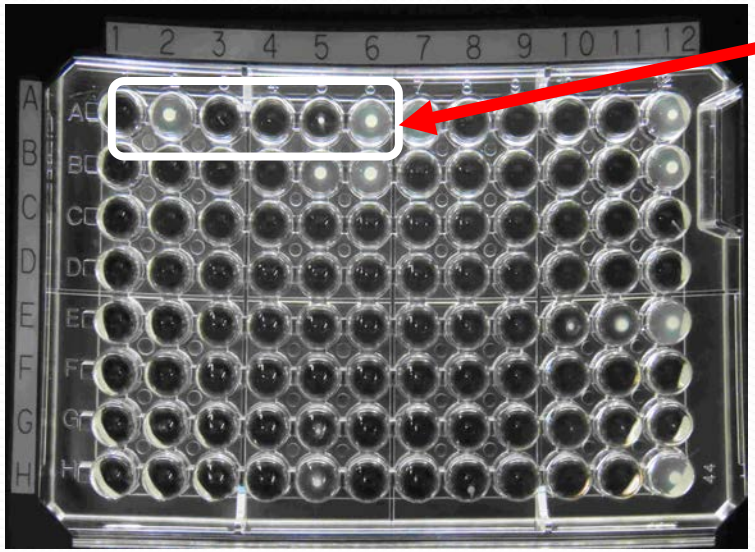
Issues in Broth Microdilution

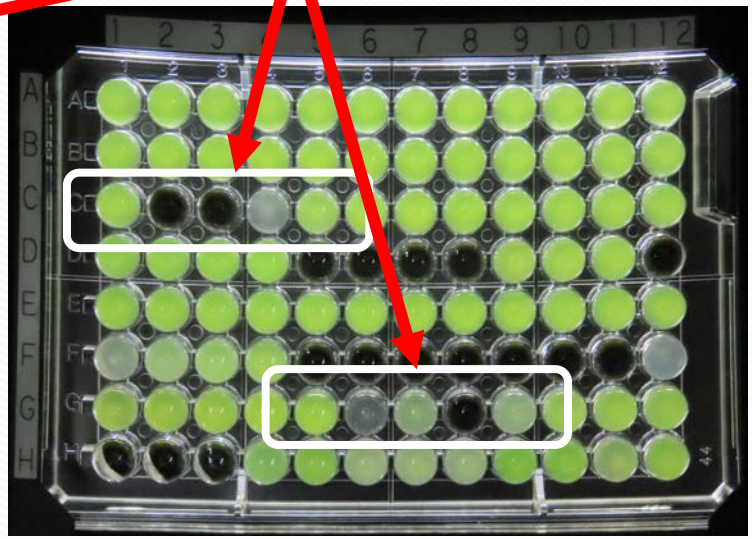
【Drug array】

	1	2	3	4	5	6	7	8	9	10	11	12
A	PIPC 64	32	16	8	4	ABPC 16	8	4	2	FOM 128	64	32
B	TAZ/PIPC 4/64	4/32	4/16	4/8	4/4	S/A 8/16	4/8	2/4	1/2	MINO 8	4	2
C	CEZ 16	8	4	2	1	0.5	CTRX 32	16	8	4	2	1
D	CAZ 16	8	4	2	1	0.5	CMZ 32	16	8	4	2	1
E	CPDX 4	2	1	GM 8	4	2	AMK 32	16	8	ST 38/2	19/1	9.5/0.5
F	CTM 4	2	1	0.5	CFPM 16	8	4	2	FMOX 16	8	4	2
G	IPM 8	4	2	1	0.5	0.25	LVFX 4	2	1	0.5	0.25	0.12
H	MEPM 8	4	2	1	0.5	0.25	0.12	AZT 16	8	4	2	Control

Skipped wells



Staphylococcus aureus Clinical Strain



Pseudomonas aeruginosa Clinical Strain

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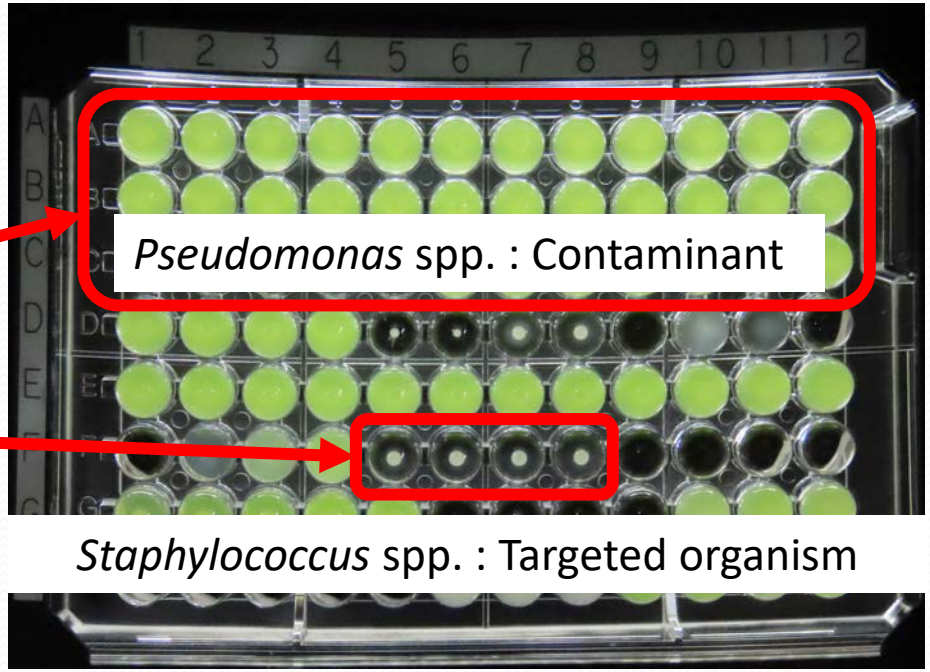
Points for Obtaining Reliable Results

- Isolate targeted organism before inoculating.
- Mix inoculum adequately.
- Perform tests based on incubation conditions.
  （agar plates, incubation temperature, incubation time, etc）

Issue 1
Failed to isolate targeted organism

2 kinds of colonies observed

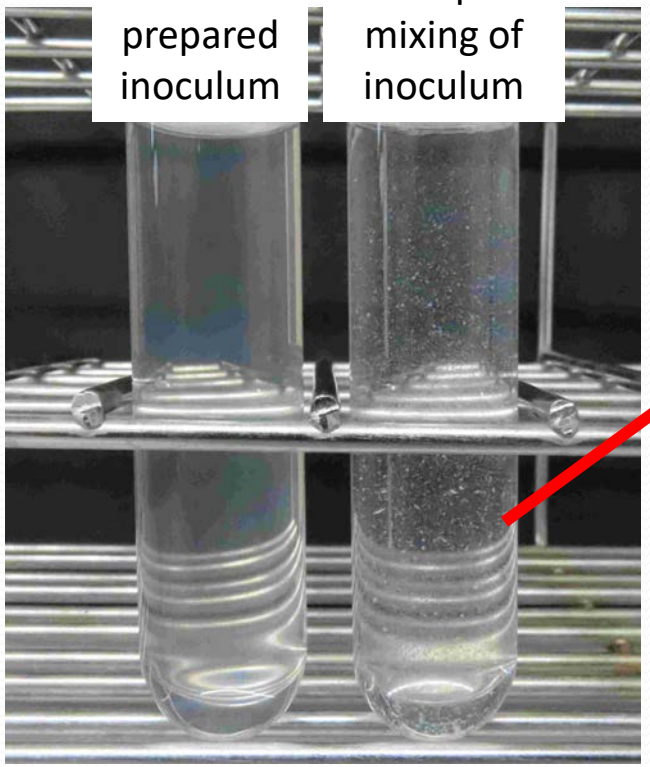
Growth of multiple organisms



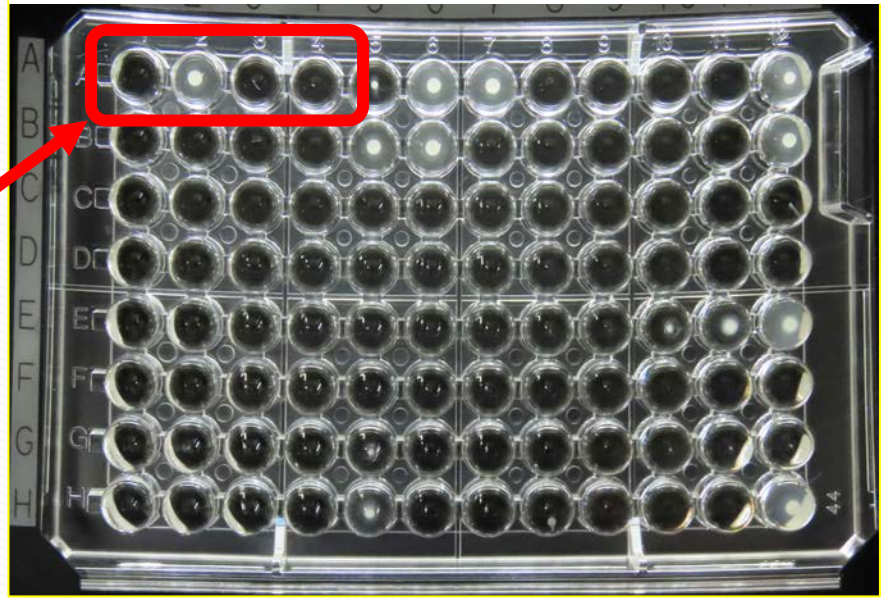
Issue 2
Irrelevant inoculum preparation

Well-prepared inoculum

Inadequate mixing of inoculum



Occurrence of skipped wells



Appropriate Test Methods

Table 2A. Zone Diameter and MIC Breakpoints for Enterobacterales

Testing Conditions		Routine QC Recommendations (see Tables 4A-1 and 5A-1 for acceptable QC ranges)
Medium:	Disk diffusion: MHA Broth dilution: CAMHB; iron-depleted CAMHB for cefiderocol (see Appendix I)[1] Agar dilution: MHA	*Escherichia coli* ATCC®[a] 25922 *Pseudomonas aeruginosa* ATCC® 27853 (for carbapenems) *Staphylococcus aureus* ATCC® 25923 (for disk diffusion) or *S. aureus* ATCC® 29213 (for dilution methods) when testing azithromycin against *Salmonella enterica* ser. Typhi *or Shigella* spp. Refer to Tables 4A-2 and 5A-2 to select strains for routine QC of ß-lactam combination agents.
Inoculum:	Broth culture method or colony suspension, equivalent to a 0.5 McFarland standard; positive blood culture broth for select antimicrobial agents with disk diffusion (see general comment [6]).	When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.
Incubation:	35°C±2°C; ambient air Disk diffusion: 16-18 hours Dilution methods: 16-20 hours	

Refer to Tables 3A, 3B, and 3C for additional testing, reporting, and QC for Enterobacterales.

※ Cited from CLSI（Clinical and Laboratory Standards Institute） M100-33rd Edition

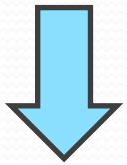
MIC variations

MIC (μg/mL)	Occurrences by Media lot			Laboratory code(occurrences)								Total
	A	B	C	A	B	C	D	E	F	G	H	
2≦												
2			1				1					1
4	21		17	10		1	14	5		4	4	38
8	63	81	67	26	29	29	23	25	28	24	27	211
16	3	5	1		1		2		2	2	2	9
32		1	1				2					2
＞32												
Total	87	87	87	36	30	30	42	30	30	30	33	261

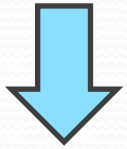
※ Cited from 2023_June_QCWG_OMN6_Presentation

Flowchart of Dried Plates Development

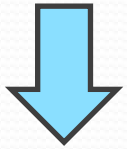
Formula Design



Industrial Process Design



Manufacturing/ Quality inspection



Dry Plate EIKEN

Solvent/diluent determination
→Performance meets CLSI QC ranges

Accuracy of dilution and solution dispensing.
Determination of drying conditions.

Standard and conformity assessment

Points for appropriate operation

- Perform tests following manufacturers' instructions. Prepare inoculum properly.
- Use panels and agar plates purchased from the same manufacture. Test QC strains if in-house agar plates are used.

Conclusion

- Broth microdilution method is widely used to measure MIC values, which provide antimicrobial information for effective treatment.
- Accuracy can be improved by operating appropriately.
- Methodological issues should also be considered.