Antimicrobial susceptibility testing (Broth microdilution method)

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Founded as OIE

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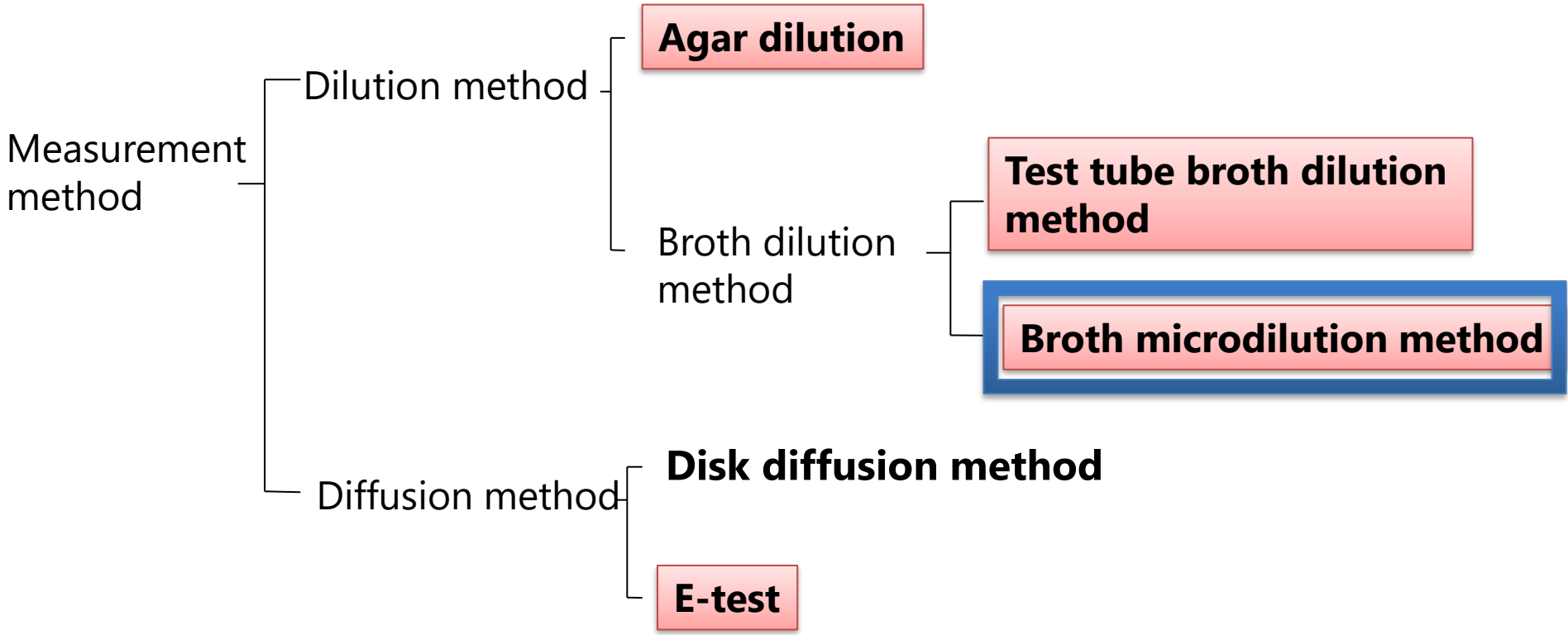
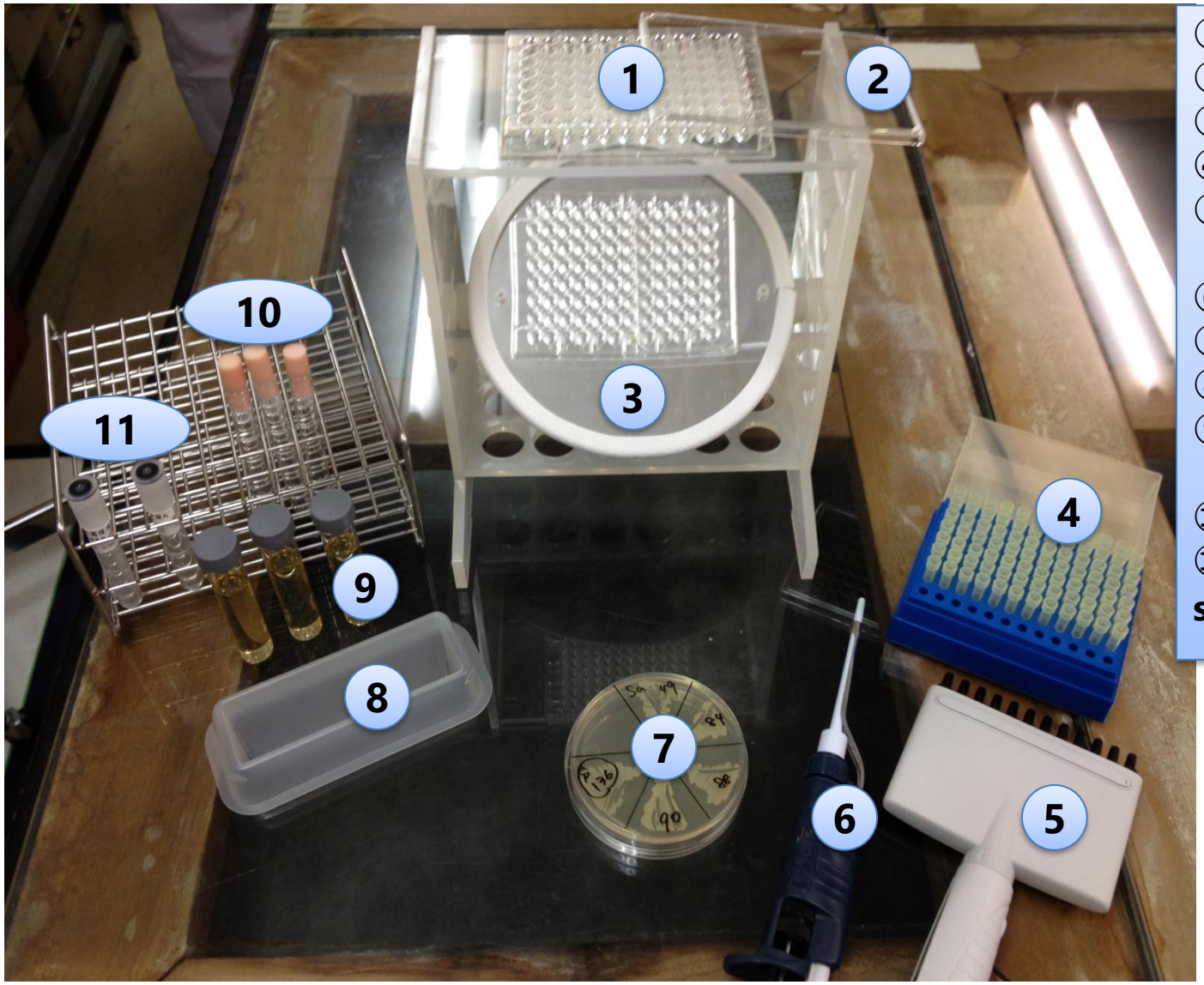
Antimicrobial susceptibility test method



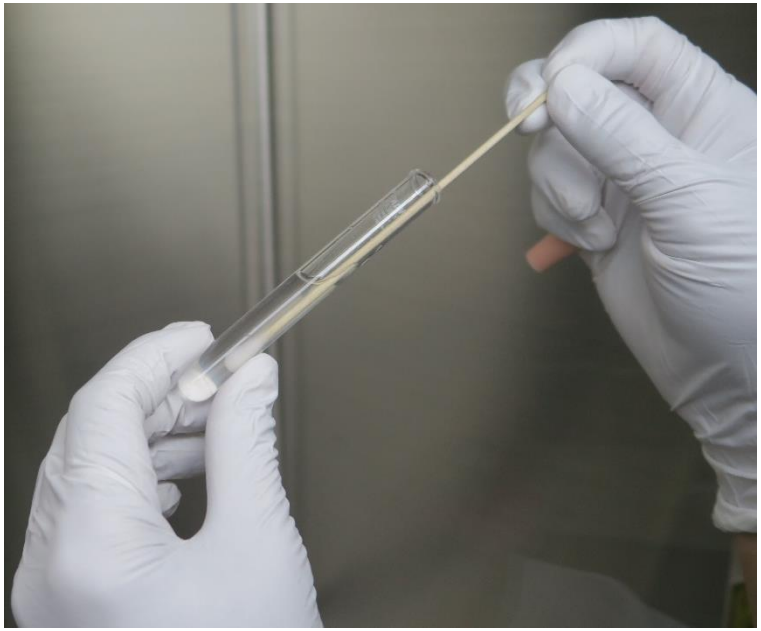
Minimum inhibitory concentration (MIC) measurement

Reagents and equipment prepared by the broth microdilution method



①Microtiter plate
②Lid
③Reading mirror
④Tip
⑤12 or 8 channel Pipette
⑥Micropipette
⑦Tested strains
⑧Reservoir
⑨Cation adjuseted Muller-Hinton Broth
⑩3 mL sterile saline
⑪Turbidity standard solution

Adjustment turbidity of bacterial suspension

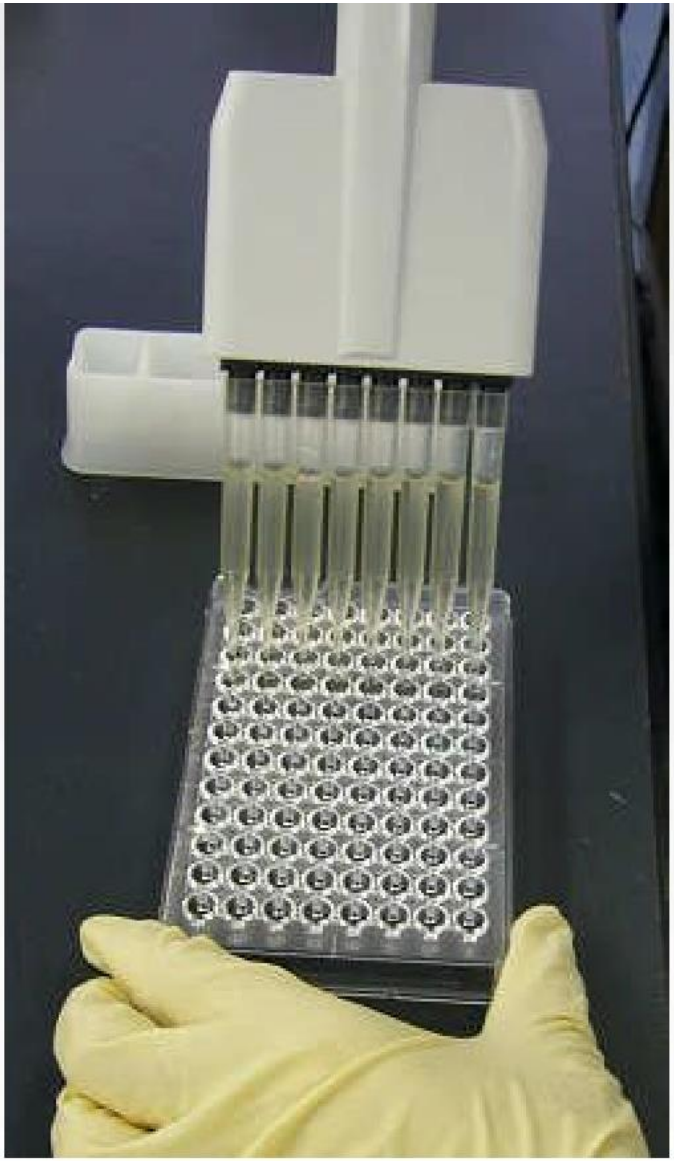


・Pick a test organism on an overnight agar culture
・With 3 mL of sterile saline suspension (Adjust the turbidity so that it becomes turbid between Mcfarland standard turbidity solution 0.5 and 1.)

Adjustment of inoculating bacterial suspension

Add 0.025 mL (25 µL) of the previously prepared bacterial suspension in ddition to 12mL Muller-Hinton broth
Mix evenly and use this as the bacterial suspension for inoculation.

Inoculation and culture of the bacterial suspension



- **Transfer inoculum into reservoir**
- **Inoculate the wells of the microtiter plate with 100 µL of the bacterial suspension using an 8- or 12-channel pipette fitted with tips, starting with the wells with the lowest drug concentration.**

  ※Remove the microtiter plate from the refrigerator beforehand and bring it back to room temperature.

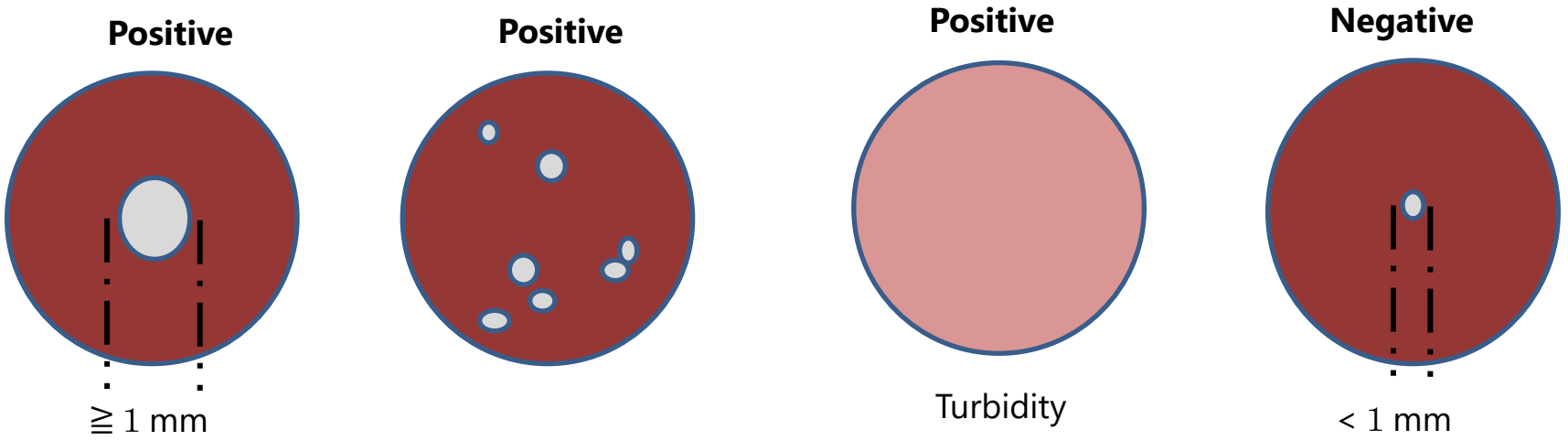
  Since the antimicrobials is dry and stuck to the bottom of the wells of the dry plate, be careful to avoid touch with the tips during inoculation.

- **After inoculation, cover the plates and incubate under the culture conditions specified for each bacterial species. (When culturing, the number of stacked plates should be limited to a maximum of three.)**

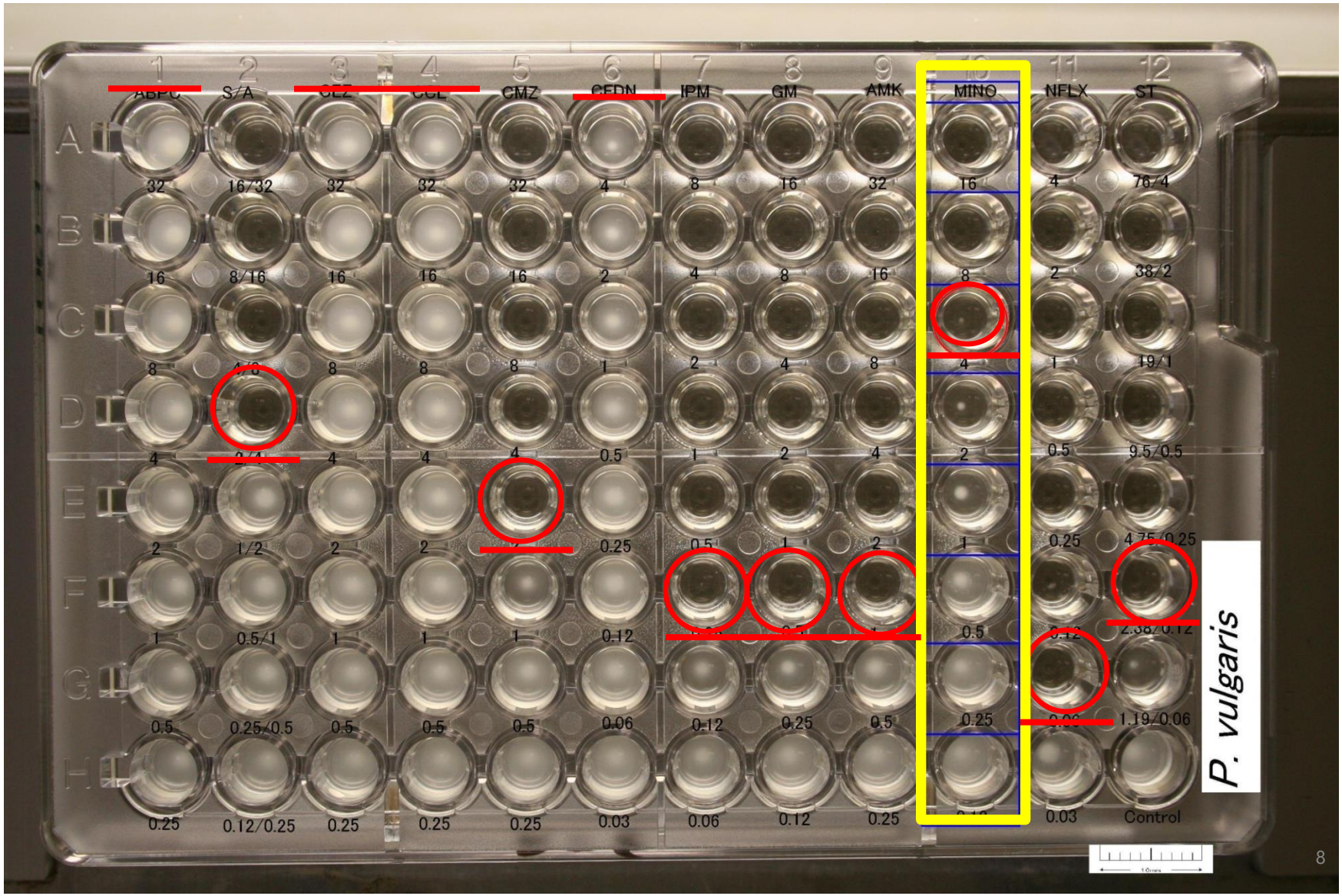
Evaluation

[Positive Growth Criteria]
・Turbidity or precipitation of 1 mm or more in diameter is observed
・Two or more precipitates are found even if the diameter of the precipitates is less than 1mm

[Negative Growth Criteria]
・No visible turbidity or precipitation
・1 precipitate less than 1 mm in diameter, even if present



Judgment example



Minimum inhibitory concentration (MIC)

	Drug dilution concentration (μg/mL)								
	0.5	1	2	4	8	16	32	64	Control
Growth of bacteria	+	+	+	+	+	-	-	-	+

MIC 16 μg/mL

	Drug dilution concentration (μg/mL)								
	0.5	1	2	4	8	16	32	64	Control
Growth of bacteria	+	+	+	+	+	+	+	+	+

MIC >64 μg/mL

	Drug dilution concentration (μg/mL)								
	0.5	1	2	4	8	16	32	64	Control
Growth of bacteria	-	-	-	-	-	-	-	-	-

MIC ≤0.5 μg/mL

Re-test required

Drug dilution concentration	Drug dilution concentration (μg/mL)								
	0.5	1	2	4	8	16	32	64	Control
Growth of bacteria	+	+	+	+	+	-	-	-	-

In the absence of control growth

Drug dilution concentration	Drug dilution concentration (μg/mL)								
	0.5	1	2	4	8	16	32	64	Control
Growth of bacteria	+	-	+	-	+	-	-	-	+

Growing (skipping) in excess of concentration

For QC strains

・When the micro dilution method is performed, the MICs of the **QC strain (according to CLSI)** are also measured.

・The MIC of the QC strain must be within the range of the QC reference value.