

Antimicrobial susceptibility testing Disk-diffusion method



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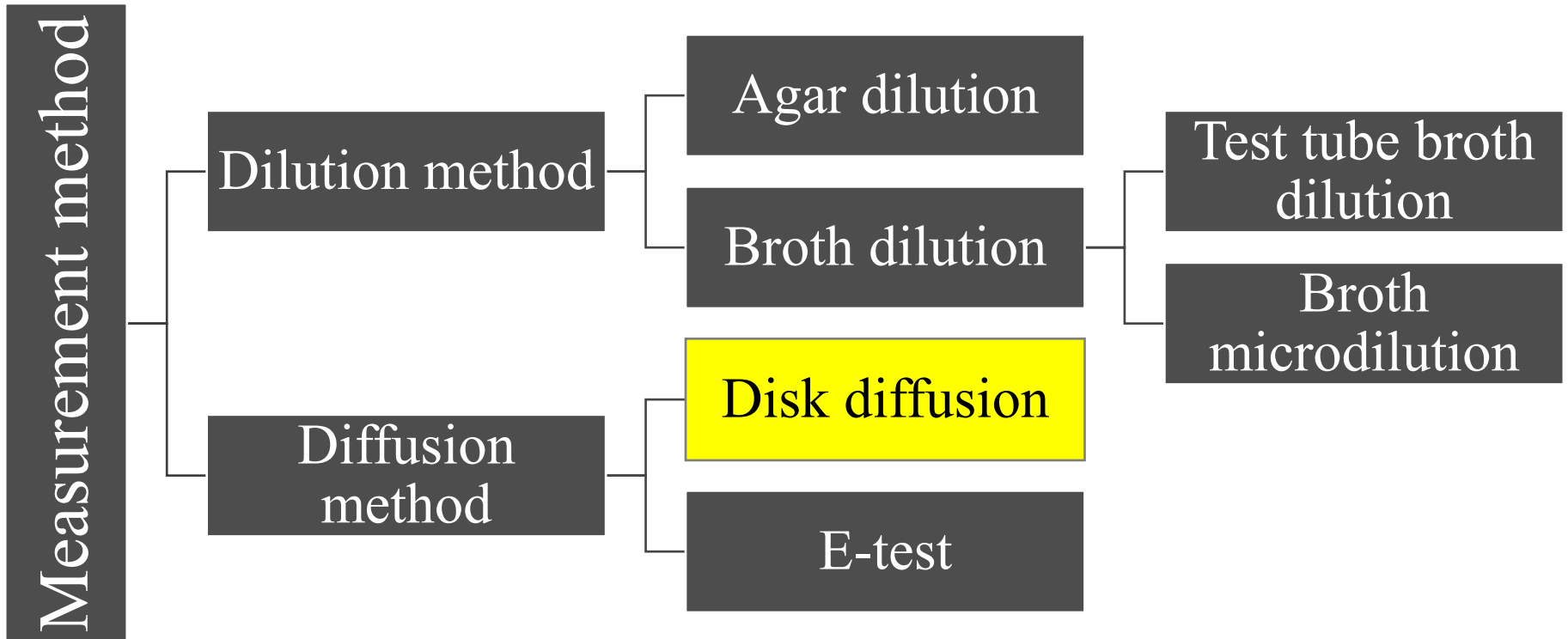
World Organisation
for Animal Health
Founded as OIE



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**Rakuno Gakuen University
Veterinary AMR Center, NVAL, MAFF**

Antimicrobial susceptibility test methods



Assess if S (susceptible), I (intermediate), or R (resistant).

Pros and cons of disk diffusion



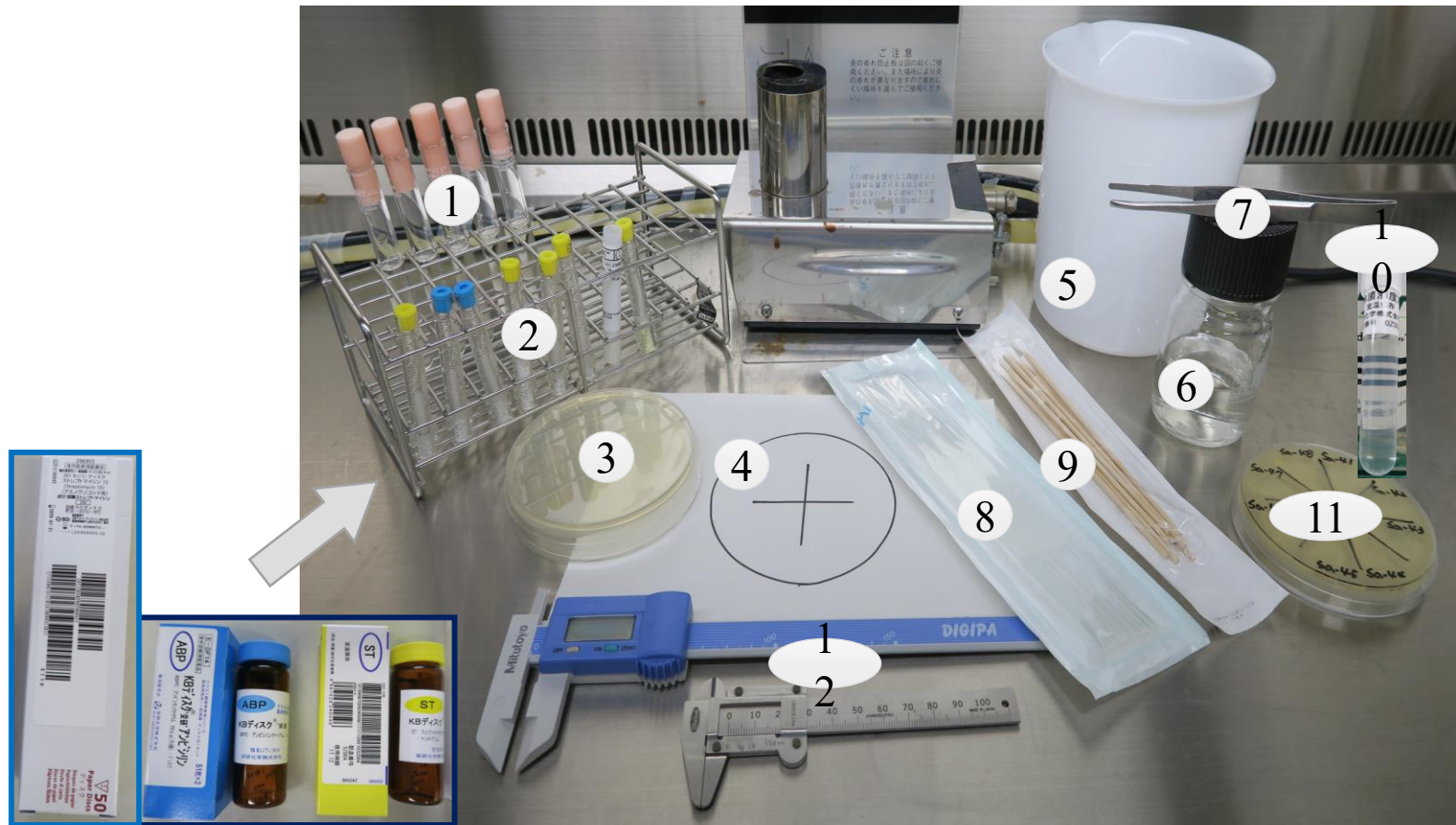
Pros

- Low cost.
- Contamination can be readily noticed.
- Can be done with commercially available materials.
- Antimicrobials are easily changeable.

Cons

- Outcome is qualitative; minimum inhibitory concentrations cannot be measured.
- Considerable effort is required to measure the inhibition zone diameter when a large number of samples are tested.

Reagents and equipment for disk diffusion method



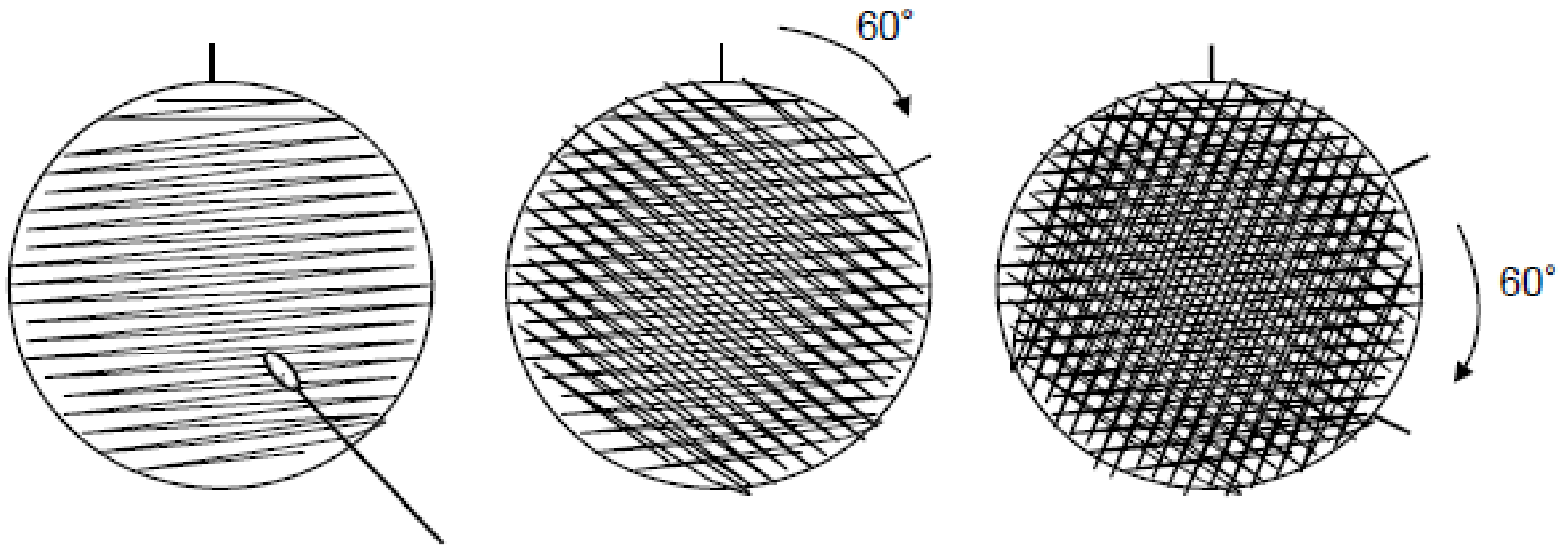
- ① 3 mL sterile saline
- ② Antimicrobial susceptibility disk
- ③ Plate for susceptibility test
- ④ Disk paper template
- ⑤ Disinfection container
- ⑥ Alcohol for flame sterilization
- ⑦ Tweezers
- ⑧ Sterile inoculation loop
- ⑨ Sterile cotton swab
- ⑩ Turbidity standard solution
- ⑪ Test bacterial strain
- ⑫ Caliper

Adjustment of bacterial suspension



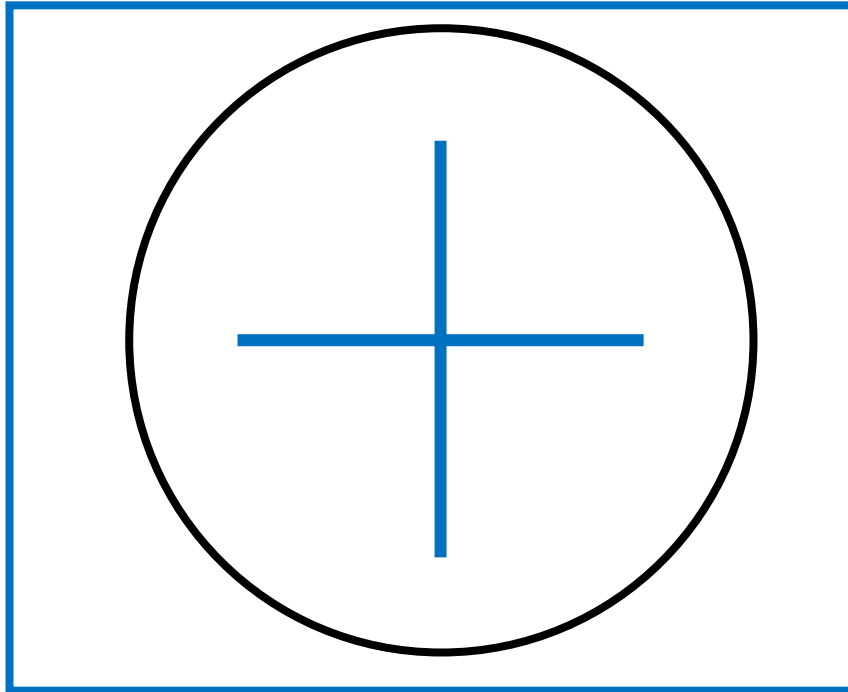
Put cultured bacteria in the saline and adjust the turbidity to 0.5 McFarland standard turbidity.

Inoculation to agar surface

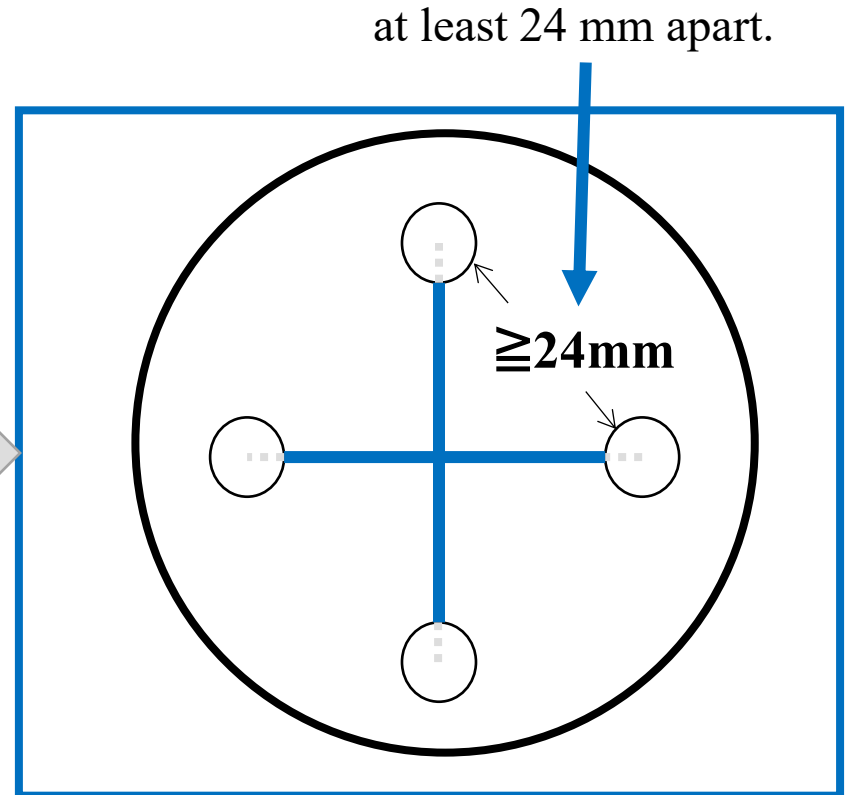
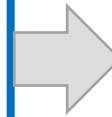


This procedure is repeated by streaking three times, rotating the plate approximately 60° each time.

Disk placement



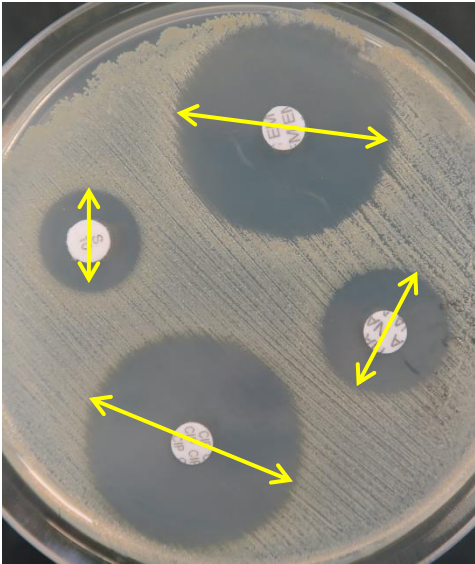
Align the plate inoculated with the test bacterium and wait for 3-5 minutes until placing disks.



With flame-sterilized tweezers, place disks where the template indicates. Ensure disks are uniformly in contact with the plate, using tweezers.

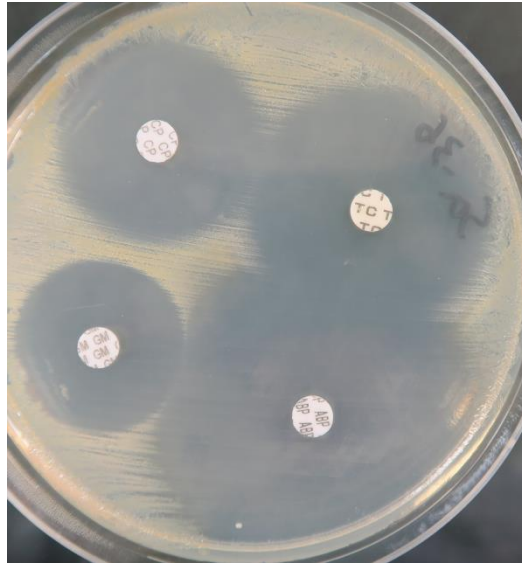
Assessment examples

①



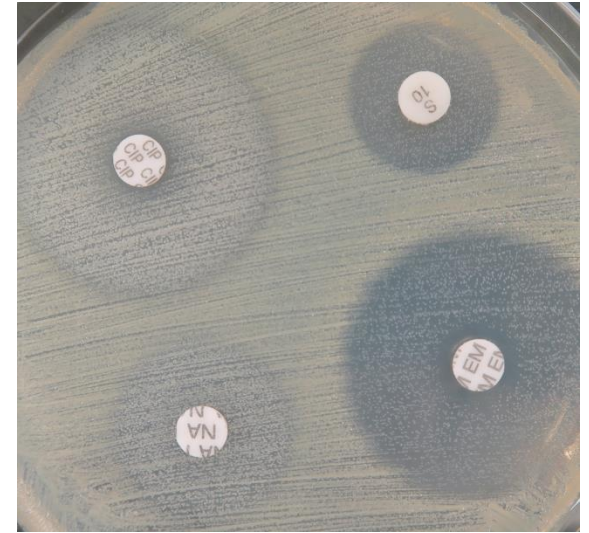
OK

②



Inhibition rings are overlapping, distorted → Change disk locations and re-test

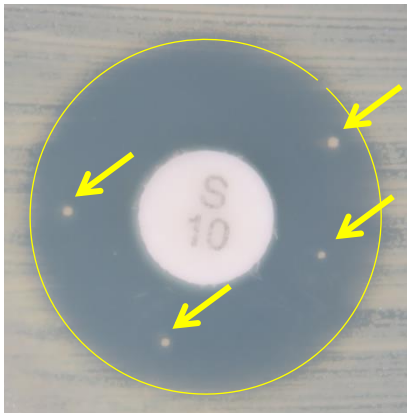
③



Contamination

→ After cloning, re-identify and test

④



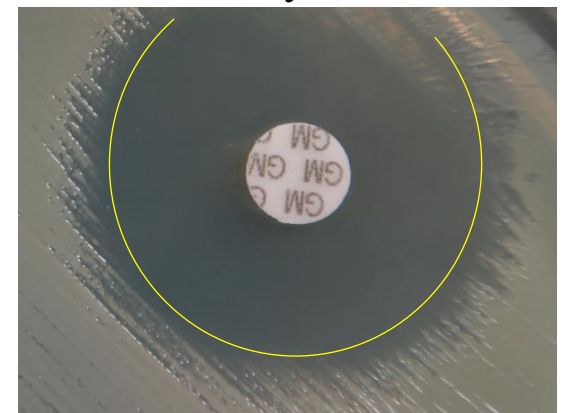
Induced resistant strains?

⑤



≤ Disk diameter (6 mm)

⑥



Measure complete inhibition ring

Quality control strains

- You are required to measure the diameter of inhibition zones for **quality control strains** in addition to the test bacterial strains when conducting the disk diffusion method.
- The first step after measurement is to check if the inhibition zone diameters for quality control strains are all within quality control reference values described in Training manual.
- **The test is considered valid when the inhibition zone diameters for quality control strains are all within quality control reference values.**