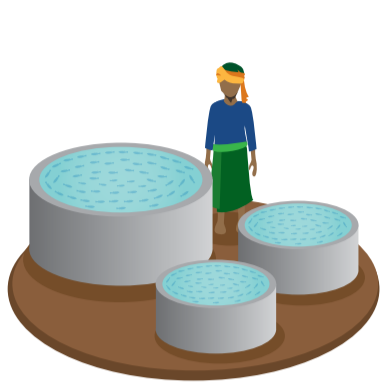


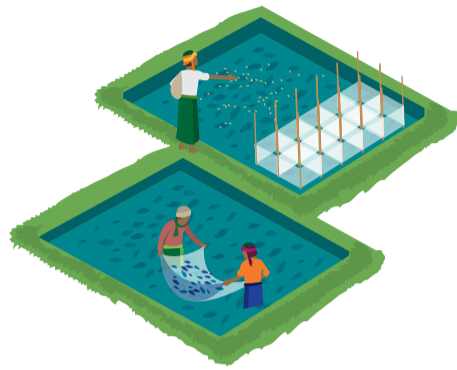
Quick protocol for antimicrobial susceptibility testing (AST) in aquatic animal species from aquaculture and fisheries

Disk diffusion method (based on CLSI guideline Vet03¹)

- 1** Collect bacteriological samples for AST from hatcheries, farms, wet markets, processing plants and environment (e.g. fish, shrimp, prawn, water, etc.). For live animals, follow standard operating protocol for euthanasia. For wet markets and processing plants, collect freshly killed animals kept on ice.



Hatcheries



Farm ponds

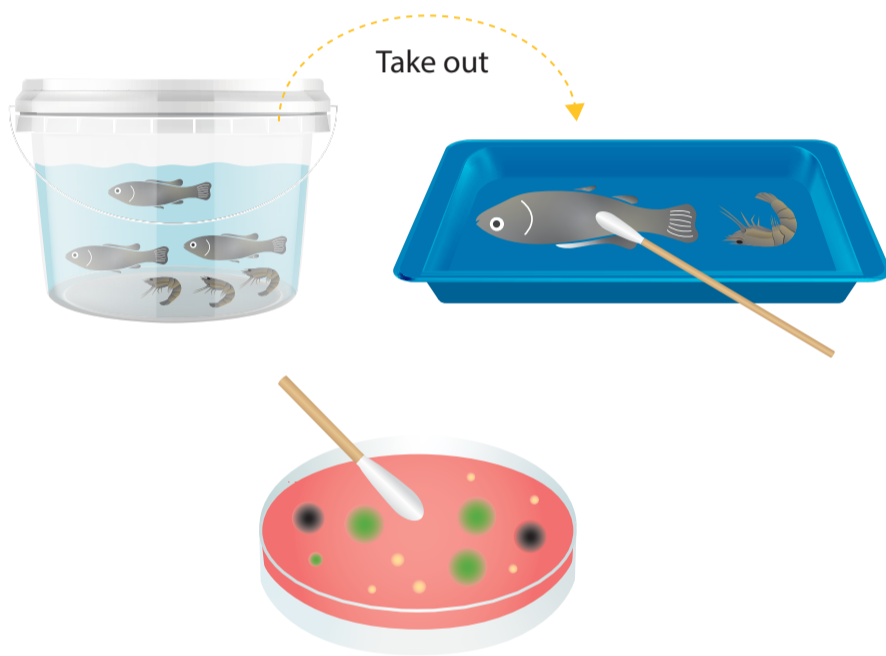


Wet markets

Preparation

- Sterilize equipment
- Ensure Muller Hinton Agar (MHA) plates are moisture free
- Remove antibiotic disks from fridge and bring to room temperature
- Run suitable reference strain alongside test samples

- 2** Bacterial isolation on appropriate media.



- 5** Inoculate 2 ml of Muller Hinton Broth (MHB)/ sterile saline with 3–5 single colonies and vortex.



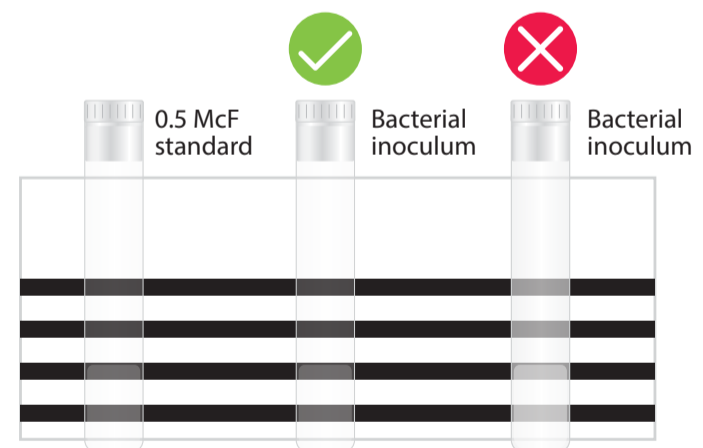
- 3** Confirm bacterial identity (e.g. gram staining, biochemical tests, molecular identification, etc.).

Note: Follow standard protocol for those tests.



Gram staining

- 6** Adjust turbidity to 0.5 McFarland.

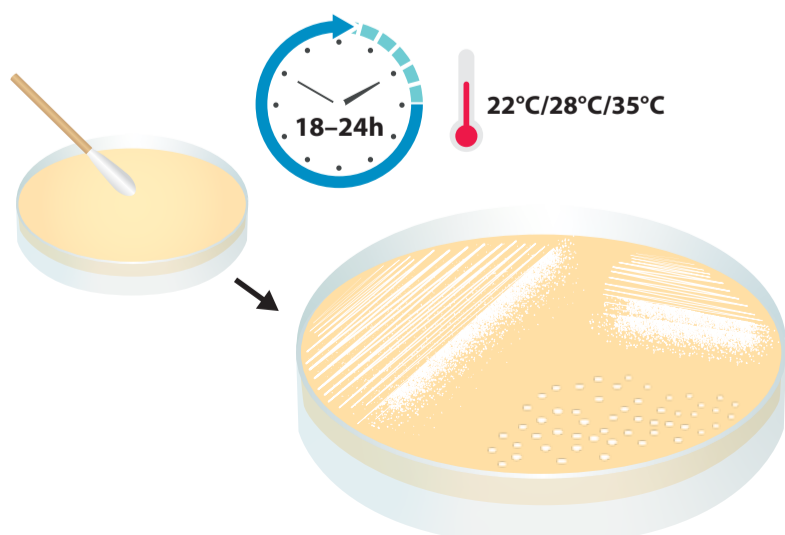


Wickerham card



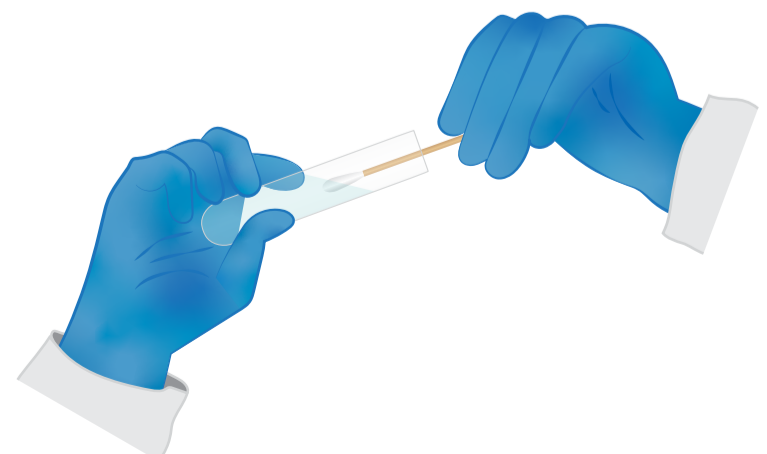
Inoculate MHA within 15 min

- 4** Incubate fresh pure colony at appropriate time/temperature (e.g. 18–24h, 22°C/28°C/35°C).

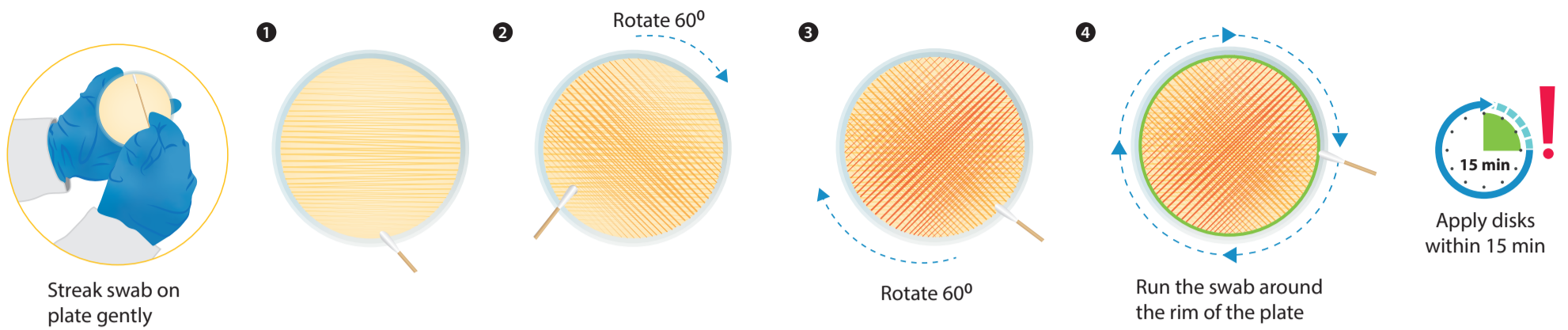


- 7** Dip sterile cotton swab in bacterial suspension.

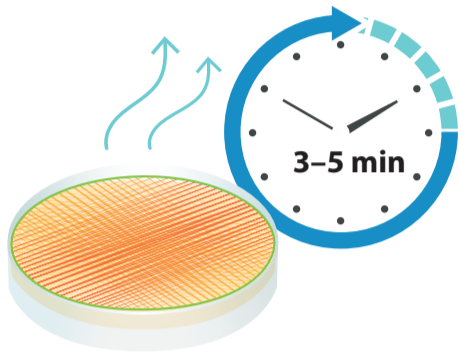
- Gram negative (-ve): Press swab against wall
- Gram positive (+ve): Leave excess on



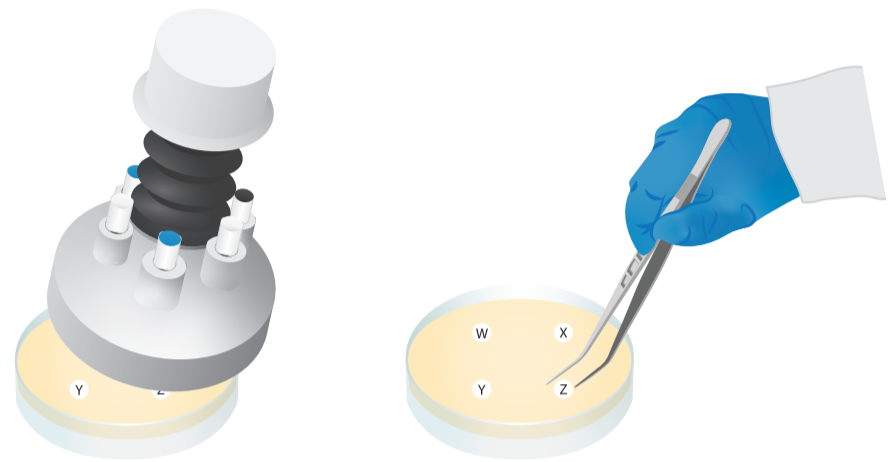
8 Streak x3 MHA plates from top to bottom. Rotate 60° and repeat. Then run the swab around rim of the plate.



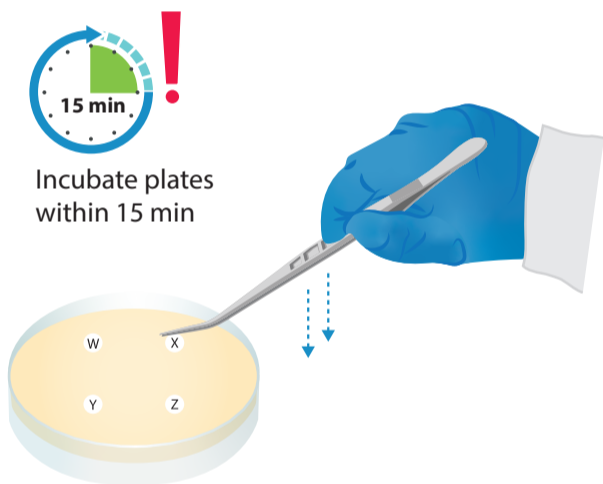
9 Allow agar plates to dry for 3–5 min.



10 Apply antibiotic disks with dispenser or sterile forceps.



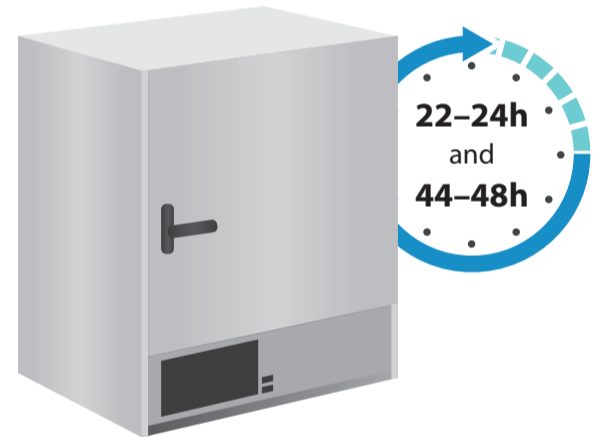
11 Gently press disks down (do not reposition).



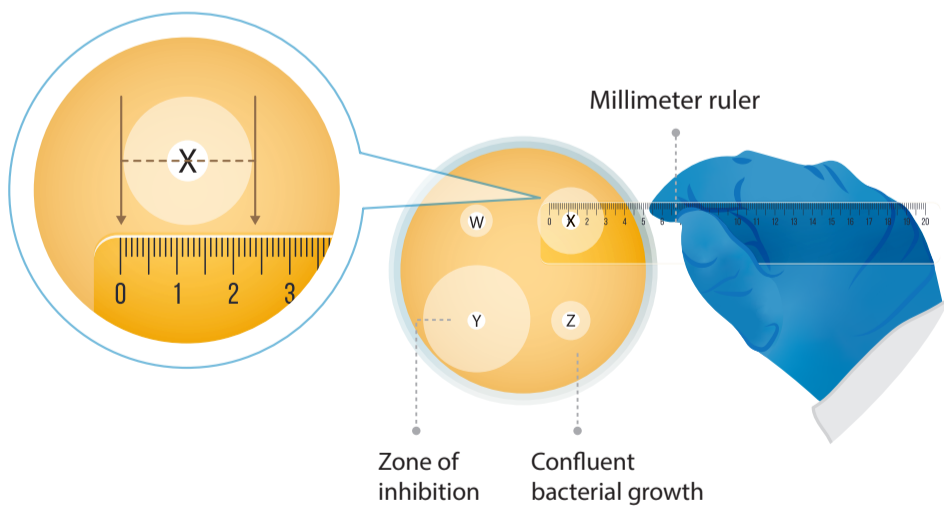
12 Incubate agar plates with disks at 22°C/28°C/35°C.



13 Read plates at recommended time for the specific isolate (typically 22–24h and 44–48h).



14 Measure inhibition zone diameter under appropriate illumination as per protocols.



15 Ensure zone sizes for reference strain are within range and determine epidemiological cut-off values (ECV/ECOFF)/clinical breakpoints if available.

| Antimicrobial agent | Disk content | Interpretive categories and zone diameter, ECVs nearest whole mm | |
|---------------------|--------------|--|------|
| | | WT | NWT |
| W | 25 µg | ≥ 27 | ≤ 26 |
| X | 30 µg | ≥ 28 | ≤ 27 |
| Y | 5 µg | ≥ 30 | ≤ 29 |
| Z | 10 µg | ≥ 18 | ≤ 17 |

* ECV, epidemiological cut-off value; NWT, non-wild type (resistant); WT, wild type (susceptible); mm, millimeter

Table 1. Example of zone of inhibition and ECV interpretation table.

Note:

¹ <https://www.clsi.org/standards/products/veterinary-medicine/documents/vet03/>