EU Reference Laboratory for Antimicrobial Resistance (EURL-AMR)
National Food Institute (DTU Food)
Technical University of Denmark

Proficiency Test 2026 for the Antimicrobial Susceptibility Testing (AST) of *Escherichia coli* and *Campylobacter*

Instructions for Reconstitution of *Campylobacter* Freeze-dried Cultures

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All strains are classified as UN3373, Biological substance, Category B.

It is the recipients' responsibility to comply with national legislation and institutional requirements for safe handling and to ensure appropriate facilities and procedures are in place.

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⚠️ Note for the Laboratories

Laboratories with **limited experience** handling crimp-sealed, rubber-stoppered serum vials with freeze-dried cultures are recommended to follow the guidance below.

✅ **Recommended approach:**

Reconstitute **through the rubber stopper** using a **sterile syringe/needle** (as described in this protocol). This approach minimizes the risk of aerosol generation, contamination associated with full metal cap removal, and accidental injury due to manipulation of the metal cap.

⚙️ **Alternative option**:

Complete **cap/stopper removal** may be performed as an alternative only by **trained personnel** using appropriate tools and safety controls.

MATERIALS FOR EACH VIAL

ITEM	DESCRIPTION
1× Crimp-sealed, rubber-stoppered serum vial with freeze-dried culture	Vials with freeze-dried cultures (**Figure 1-2**)
Sterile forceps	To open the metal flip-off disc of the metal cap of the vial
0.5 mL + 5 mL Non-selective Broth	Non-selective broth suitable for *Campylobacter*
1× Non-selective Agar Plate	Non-selective agar suitable for *Campylobacter*
1× 1 mL Sterile Syringe (0.01 mL graduations preferred) & 1× Sterile Needles (21–23 G)	For reconstitution and withdrawal through the stopper (**Figure 3**)
1× Sterile Vent/air needle (21–23 G) - *Optional*	To equalize pressure (recommended for ease/safety; not required) (**Figure 3**)
Sharps container	For immediate disposal of needles
70% ethanol	Disinfection

EQUIPMENT

ITEM	DESCRIPTION
LAF-bench	Aseptic conditions
Bunsen burner, if LAF-bench unavailable	Aseptic conditions
Microaerophilic system	3–5% O_2 and 10% CO_2
Inoculation loops	For plate inoculation
41.5°C or 37°C incubator	Standard incubation

SAFETY & SETUP

1. Wear appropriate PPE (lab coat, gloves, eye protection).

2. Handle UN3373 Biological Substance, Category B materials in a **Class II biosafety cabinet** (BSL-2 equivalent).

3. Maintain **aseptic technique** at all times. Work in LAF-bench or close to a Bunsen burner.

BEFORE STARTING

4. Bring in the LAF-bench, or other designated working area, the following materials:
   - Vial(s) with freeze-dried culture(s)
   - Sharps container for immediate disposal of needles
   - Sterile 1 mL syringes (0.01 mL graduations preferred) and 21–23 G needles for reconstitution and withdrawal through the stopper
   - Vent/air needle (optional)
   - Agar plates (prewarmed at 37°C)
   - Tube with pre-warmed (37 °C) reconstitution broth (0.5mL per vial)
   - Tube with 5mL pre-warmed (37 °C) broth per vial (backup culture)
   - 70% Ethanol

5. Identify the vial parts (metal flip-off disc, metal cap, rubber stopper) shown in **Figure 1**.

6. Gently tap the vial so the freeze-dried cake settles at the bottom.

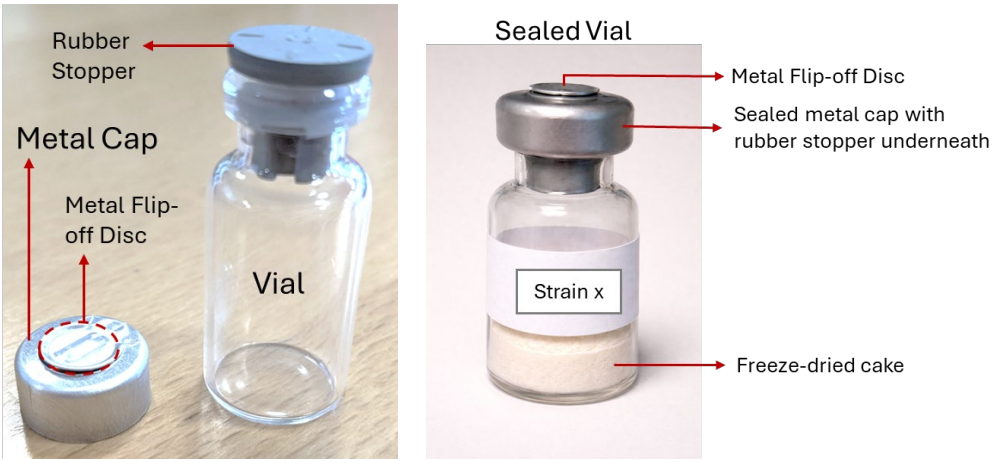

Figure 1. **Components and final configuration of a serum vial used for freeze-dried bacterial cultures**. **Left**: individual vial components, including glass vial, rubber stopper, metal cap, and flip-off disc. **Right**: assembled and sealed vial containing a freeze-dried cake at the bottom, with the rubber stopper secured by a crimped metal cap with a flip-off disc.

PROCEDURE

7. Disinfect the vial's exterior with 70% ethanol and let dry.

8. Using sterile forceps, pop off the metal flip-off disc:

9. Wipe the exposed rubber stopper with 70% ethanol and let air-dry 30 s.

10. (**Optional**) Insert the vent/air needle through the rubber stopper to gently equalize pressure (keep the tip in headspace, above the cake) (**Figure 3**).

11. With a sterile 1 mL syringe + 21–23 G needle, slowly (to avoid splashing) inject 0.5 mL of pre-warmed (37 °C) broth through the rubber stopper. **(!)** With one hand holding the vial steady on the bench, use the other hand to hold the syringe barrel securely. Don't let the syringe tip move or twist inside the rubber stopper while the liquid is being injected.

12. Mix by gently swirling the vial or by slowly aspirating/dispensing with the same syringe 3–5× until the cake is fully dissolved. **DO NOT REMOVE THE SYRINGE FROM THE VIAL.** Withdraw all the suspension (use 0.01 mL graduations if available).

13. Remove the syringe from the vial and **immediately** dispense:

a. Approximately 10µL onto a pre-warmed (37 °C), non-selective agar plate (**primary culture**)
b. The remaining suspension into 5 mL of pre-warmed broth (**backup culture**)

Streak the droplet on the agar plate with a sterile inoculation loop using a standard technique.

14. Incubate the agar plate and the broth culture immediately under microaerophilic conditions (3–5% O_2, 10% CO_2) at 41.5 ± 1°C for 24 ± 2 h, or alternatively at 37 ± 1°C for 24-48 h. **(!) Keep tube caps loose of the liquid culture for gas exchange**.

EXPECTED RESULTS & TROUBLESHOOTING

15. Adequate growth should be visible on the agar plate after the stated incubation.

16. If **no colonies appear after 48 h**, check the broth culture for growth and re-streak fresh agar from the broth culture.

PREPARATION FOR BROTH MICRODILUTION & LONG-TERM STORAGE:

(!) Do not prepare the broth microdilution (BMD) inoculum or long-term storage cultures directly from the primary recovery plate following reconstitution of the freeze-dried culture.

Following initial recovery, prepare **a fresh subculture** on non-selective agar suitable for *Campylobacter* and use growth from this fresh subculture plate for BMD testing and preparation of long-term storage cultures (*e.g.*, in glycerol-containing cryopreservation medium at −80 °C) according to the laboratory's internal procedures.