PROTOCOL FOR LONG-TERM STORAGE OF *CAMPYLOBACTER*

Version 1

Publication history

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Context

To ensure harmonisation of the antimicrobial resistance (AMR) monitoring of *C. jejuni* and *C. coli* from food-producing animals within the EU as set out in Commission Implementing Decision 2020/1729, the use of a harmonised protocol [1] is recommended by EFSA, the EURL for antimicrobial resistance (EURL-AR) and the European Commission.

Commission Implementing Decision 2020/1729 requires that resistant isolates shall be stored by the Member State laboratories at a temperature of – 80 °C for a minimum period of five years. Other temperatures of storage may be used provided that they ensure viability and absence of changes in strain properties.

Scope of the method

This protocol describes recommendations and practical solutions for the preparation of *Campylobacter* strains for long-term storage. It should be considered a complement to the harmonised protocol for isolation, identification and storage of *Campylobacter jejuni* and/or *C. coli* for the EU monitoring of antimicrobial resistance [1].

Protocol

There are five key-points in the preparation of *Campylobacter* strains for long-term storage, included in 3.1-3.4 below.

3.1 Purification

The purpose of purification is to avoid analysing and storing a mixed culture of contaminating background flora and or several *Campylobacter* strains. Performing two purification steps on non-selective blood agar plates is recommended. When doing the first purification, pick a typical or suspect colony and make a three or four-way streak on a non-selective blood agar plate to obtain single, well-isolated colonies. Use an inoculation loop to do the streaks and burn or switch inoculation loop between streaks. When doing the second purification, pick a single, well-isolated colony and make one dense streak on a new non-selective blood agar plate to obtain a heavy growth of a pure culture for identification and storage. Check that the plate looks pure before harvest for storage, otherwise repeat the purification step. Incubate the non-selective agar plates in a microaerobic atmosphere at 41,5 °C +/-1°C for 24−48 hours. The second purification plate, intended for storage, should be incubated until a dense culture. Plates can, if necessary, be stored refrigerated in microaerobic atmosphere over the weekend (i.e. for up to 72 hours). However, it is recommended to use as fresh culture as possible for storage.

3.2 Purification

Harvest the entire lawn of bacteria on a full plate for storage and transfer to beads or liquid storage media. If liquid storage media is used, make sure to thoroughly homogenise the suspension of bacteria in the storage

media to avoid an uneven distribution (lumps) of bacteria.

3.3 Storage media

For storage in liquid media, use a nutrient broth supplemented with glycerol (at least 15%) as cryoprotectant. The storage media can be further improved by supplementation of FBP [2] (also known as *Campylobacter* growth supplement and includes sodium pyruvate, sodium metabisulfite and iron sulfate hydrate), defibrinated, lysed or laked blood [3], or serum.

3.4 Storage temperature

Cryovials with bacteria prepared for long-term storage should be transferred to storage at - 70 °C or colder on the same day as they are prepared. Intermediate storage at - 20 °C for more than a few hours should be avoided [2, 3].

3.5 Recovery of strains from storage

Keep cryovials on ice or in a cooling rack during the laboratory work. Avoid allowing the liquid cultures to thaw. Scrape a small amount, at least the size of a grain of rice, of the inoculum or one bead, and streak on a non-selective blood agar plate. If no or poor growth from the initial streak of the frozen stock, inoculate new plates with a larger amount of the inoculum.

4. References

[1] EURL-*Campylobacter*. 2020. Protocol for isolation, identification and storage of *Campylobacter jejuni* and/or *C. coli* for the EU monitoring of antimicrobial resistance. https://www.sva.se/media/0dbccrjh/harmonised-protocol-campy-for-amr-mon-version-1-final_2.pdf

[2] R. GORMAN AND C.C. ADLEY. 2004. An evaluation of five preservation techniques and conventional freezing temperatures of 20°C and 85°C for long-term preservation of *Campylobacter jejuni.* https://doi.org/10.1111/j.1472-765X.2004.01490.x

[3] O'la AL-Fawares et al. 2023. Comparison of preservation enrichment media for long storage duration of *Campylobacter jejuni*. https://doi.org/10.7845/kjm.2023.3058