

Proficiency Testing for the enumeration of *Campylobacter* spp. in food

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INTRODUCTION

REQUASUD is a Belgian Proficiency Testing (PT) provider. The food microbiology PT schemes currently involve 29 laboratories. The parameter "enumeration of *Campylobacter* spp. in fresh ham samples" was validated and introduced in REQUASUD's PT in 2012.

Out of 10 *Campylobacter* spp. strains tested, two *C. jejuni* were characteristic and robust enough to be used in PT schemes: C3, a reference strain, and E3, isolated from a food intoxication. However, when inoculated into ham sausage, homogenized and distributed under refrigeration, inactivation of 99,9 % of the inoculum was observed, which resulted in:

- A low assigned value (close to the quantification limit)
- A high dispersion of the results ($s_R = 0,68$), as illustrated in Fig. 1

In 2020-2021, REQUASUD conducted investigations to:

- Improve *Campylobacter* spp. survival in PT samples by an optimized inoculation protocol
- Quantify the *Campylobacter* inactivation at each step of the sample preparation

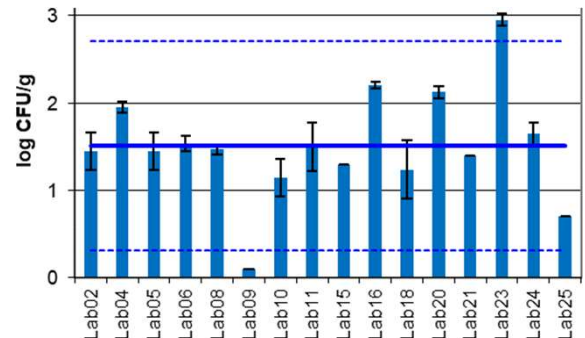


Fig 1. Results of the enumeration of *Campylobacter* spp. in two ham sausage samples during PT2019-1
— = assigned value ; --- = confidence interval

MATERIAL AND METHODS

OPTIMIZATION OF THE INOCULATION PROTOCOL

The first purpose was to improve the survival of strains C3 and E3 in ham sausage samples, by comparing different inoculation procedures and media.

- Culture media: Rapid*Campylobacter*, mCCDA, PCA, Horse Blood Agar *
- Incubation times/temperatures: 24-72h and 37-44°C in micro-aerobiosis
- Diluents for liquid inocula: BPW, BPW + 30% glycerol, thioglycolate **

* Blood agar (Fig. 2) provides essential cofactors to promote *Campylobacter* growth and survival

** The diluent thioglycolate broth (Fig. 3) protects oxygen-sensitive bacteria during inoculation

STEPS INDUCING THE HIGHEST INACTIVATION

The second purpose of this study was to determine which steps of the ham sample preparation (homogenization in a lab blender, or storage at 4°C) induced the highest inactivation of the strains.

The *Campylobacter* spp. were enumerated five times in each ham sample: at the time of inoculation, after 30 and 90 sec of mixing and after 24 and 48h of storage at 3°C.

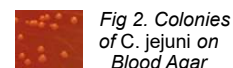


Fig 2. Colonies of *C. jejuni* on Blood Agar

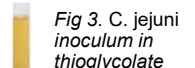
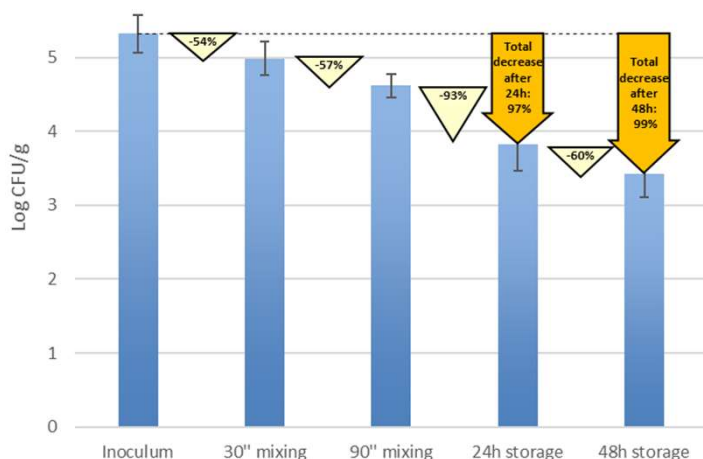


Fig 3. *C. jejuni* inoculum in thioglycolate

RESULTS AND DISCUSSION



The survival of the two *Campylobacter* spp. inocula into ham samples was improved by the following optimized protocol:

- Strain growth on Blood Agar (48 h at 41,5°C, micro-aerobiosis),
- Suspension into thioglycolate broth,
- High-level inoculation into ham sausage ($> 10^4$ CFU/g),
- Mixing, to ensure sample homogeneity (90 sec),
- Storage for maximum 24 h at 3°C.

Although these technical adaptations improved the strain survival, a decrease of about 1.5 log was still observed in the PT samples after 24 h (Fig. 4). Globally, 97% of the initial inoculum was "lost" after homogenization and storage of the samples.

When the samples were stored 48 h instead of 24 h, we observed a 60% additional inactivation, conducting to a total loss of 99% of the initial inoculum.

Fig. 4. Mean inactivation of *Campylobacter* C3 and E3 inocula during homogenization and storage of the PT samples (when using the optimized inoculation protocol).

CONCLUSIONS

The parameter "Enumeration of *Campylobacter* spp. in food samples" represents a technical challenge for food microbiology PT providers due to the low survival of this micro-aerophilic and fastidious bacterium. Thanks to a comprehensive study of the factors affecting the *Campylobacter* spp. inactivation (i.e. need for essential cofactors, protection towards oxidation during mixing, limited storage time), REQUASUD managed to organize PT, including currently 19 laboratories, for this parameter in fresh ham sausage samples.

Yet, some technical constraints remain:

- Seen the 97% inactivation, the initial inoculation level must be higher than 10^4 CFU/g to guarantee a final level above the quantification limit;
- Samples can be stored for maximum 24 h at 3°C, making such PT schemes difficult to export to foreign countries.