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Evaluation of a chromogenic media for the rapid enumeration of Bacillus ceureus sensu lato in 24 hours, without confirmation.

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Bacillus cereus sensu lato (*B. cereus*) enumeration using traditional methods such as ISO 7932 and AOAC 980.31 may be challenging due to the low selectivity of the Mannitol egg yolk polymyxin agar (MYP), and the multiple confirmation steps that can take up to 5 days to confirm results. The RAPID'*B.cereus* chromogenic media (RBC) protocol is an alternative method validated by AFNOR for *B. cereus* enumeration in 24 +/- 3 hr, on all human food products, animal feed products and environmental samples of industrial production, without confirmation. Fast methods with high selectivity are extremely important to the food industry, considering the daily high volume of tests and the need to reduce time to results. This study aimed to evaluate the performance of the RAPID'*B.cereus* protocol to enumerate *B. cereus* on meat extract samples compared to the standard methodology. The tests were performed with thirty meat extract samples (25 g) randomly collected from the industrial laboratory during routine analyses of a Brazilian beef producer. Meat extract was chosen as a challenging matrix, as it may contain damaged bacterial cells due to thermal processing and was not included in the AFNOR validation study scope. Samples were split into two groups and analyzed separately. The first group (n=20) was designed to evaluate the natural sample contamination and the media capability to inhibit natural background flora. Samples were diluted 10-fold in Buffered Peptone Water (BPW) and plated on the surface of RBC and MYP. After incubation (30°C for 24 hr), typical and atypical colonies were counted on both media for comparison. In the second group test, ten samples previously confirmed as negative using the AOAC 980.31 standard method, were contaminated with 10^2 – 10^3 CFU g^{-1} of *B. cereus* ATCC 11778. Samples were diluted 10-fold in BPW and plated on RBC and MYP by two analysts. After incubation (30°C for 24 hr), typical colonies were counted. The methods\' reproducibility was estimated and compared using the F test ($\alpha = 0.05$). One-way analysis of variance (ANOVA) ($\alpha = 0.05$) was used to compare the analyses between media. Samples with natural contamination showed *B. cereus* close to the minimum detection limit of the plating technique in both media (1 log CFU.g^{-1}), not allowing statistical comparison. However, the selectivity of RBC was proven to completely inhibit the growth of background flora on the plates. In MYP, atypical colonies were identified in 14 of the 20 tested samples, with counts ranging between 1.0 and 1.9 log CFU g^{-1}. The presence of atypical colonies can modify the MYP medium characteristics, making it hard to identify and enumerate the target colonies, reducing the reliability of the method. The spiked samples presented counts varying between 2.0 and 2.8 log CFU g^{-1}, considering both culture media and analysts.

The standard deviations of intralaboratory reproducibility, were estimated at 0.27 and 0.22 for MYP and RBC, respectively, showing no significant statistical differences. In addition, no significant differences were observed between MYP and RBC in the recovery of *B. cereus* strains from inoculated samples. The RBC alternative method is reliable for the enumeration of *B. cereus* in meat extract samples and offers a more user-friendly protocol than MYP agar. In addition, the RBC protocol may bring a reduction of at least four days in time to results in relation to the AOAC 980.31 standard method.

Agradecimentos: