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Detection of Salmonella spp. in meat products: A 48 hr solution from enrichment to confirmation using qPCR automation and chromogenic media.

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Food pathogen detection workflows include enrichment, screening and confirmation steps. Traditional methods such as ISO 6579-1:2017 for *Salmonella* detection can be time consuming (up to seven days) and require multiple culture media, decreasing laboratory productivity and increasing costs. Fast methods that simplify this process and reduce time to results, bringing more sensitivity and selectivity, are essential for the routine of industrial laboratories. This study evaluated a complete solution for detection and confirmation of *Salmonella* spp. in 48 hr, using an automated real-time PCR method followed by plating in selective chromogenic media and latex confirmation. Sixty-three samples of meat and bone meal (25 g) known to be negative for *Salmonella* were inoculated with *Escherichia coli* (1000 CFU/sample), chosen as interferent. Of these, thirty samples were additionally inoculated with *Salmonella* Typhimurium (10 CFU/sample). Meat and bone meal were chosen as a challenging matrix, as they contain high levels of qPCR inhibitors and background flora that can hinder the recovery of the target bacteria. Samples were diluted 10-fold in BPW and enriched at 37 ± 1°C for 22 hr. DNA extraction and qPCR plate set up were performed by the iQ-Check Prep Automation System, using the iQ-Check *Salmonella* II Kit. PCR runs were performed using the FAST thermal protocol on a CFX96 Touch Deep Well Real-Time PCR Detection System. After screening, ten positive and ten negative samples were randomly chosen and plated (10 µl) on the surface of RAPID'*Salmonella* chromogenic media directly and after secondary enrichment in RVS broth (41,5 ± 1°C for 22 hr). Additionally, samples were also plated onto XLD for comparison with the chromogenic media. All plates were incubated at 37 ± 1°C for 22 hr. Typical colonies were picked from each media and confirmed using *Salmonella* latex tests. Results were analyzed considering sensitivity, specificity, precision, false positive and false negative rate, according to DOC-CGCRE-089 (2017) from the INMETRO General Accreditation Coordination. The combined solution of qPCR and chromogenic media proved capable of detecting the pathogen in all inoculated samples, before and after secondary enrichment. This method brings a time to results saving of at least 3 days for positive samples in comparison to the ISO 6579-1:2017 reference method. Even analyzing challenging samples, no qPCR or bacteria growth inhibitions were found, and the results showed sensitivity, specificity, and precision of 100%, without false positive or false negative results. On the other hand, XLD plating gave a false positive result confirmed according to ISO 6579-1. Other *Enterobacteriaceae* also have the capacity to produce H_2S, which may explain the presence of colonies that look like *Salmonella* on XLD. Additionally, the pathogen recovery was higher with RAPID'*Salmonella* compared to XLD, indicating a greater ability of the

chromogenic media to inhibit background flora in real samples. This study demonstrated the effectiveness of the complete solution for *Salmonella* detection in less than 48 hr. The power of automation associated with the sensitivity of qPCR and the selectivity of the chromogenic media proves to be a potent tool for industrial laboratories, shortening time to results and operational steps, and improving the laboratory profitability as well as analysis reliability.

Agradecimentos: