ISOLATION AND IDENTIFICATION ANALYSIS METHOD FOR
Salmonella Typhimurium IN MILK USING REAL-TIME
PCR (Polymerase Chain Reaction)

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ABSTRACT

Salmonella Typhimurium is one of pathogenic bacteria that cause foodborne disease. Conventional culture methods which is traditionally been considered as a “gold standards” are a common method to identified this pathogen. However, conventional culture methods are labor-intensive and time-consuming. This study aimed to obtain a rapid and precise analytical method for Salmonella Typhimurium, to compare boiling method and commercial kit method using QIAamp® DNA Blood Mini Kit for extraction of DNA, identify and quantify Salmonella Typhimurium on sterilized milk using real-time PCR. Stages of this research are doing enrichment in pure culture and spiked sample, isolating the DNA of Salmonella Typhimurium by boiling method and commercial kit method, and then running a real-time PCR for determining specificity of the primers, determining primers concentration and doing quantification of Salmonella Typhimurium in sterilized milk. Commercial kit method produces DNA template purer than boiling method. So it affects their efficiency value. Commercial kit method assay has better efficiency than boiling method assay. It is 100% for commercial kit method and 386.8% for boiling method. The concentration of primers which is used in real-time PCR assay is 0.125 μM and its primers are specific for Salmonella Typhimurium. The result of Salmonella Typhimurium quantification in sterilize milk are not the same as quantification result from conventional method. But it has the same result with concentration value of Salmonella Typhimurium that is added to sterilize milk which is counted by Petroff-Hausser.

Keywords: real-time PCR, Salmonella Typhimurium, boiling method, QIAamp® DNA Blood Mini Kit, milk