P78

Design of Internal Amplification Control for PCR Detection of Salmonella typhi

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Introduction: Salmonellae have general characteristics of Entrobacteriaceae family. Diagnostic methods for detecting this microbe which routinely used in laboratories are time-consuming and are not hundred percent reliable.Molecular methods such as PCR are new and powerful techniques that provide more accurate diagnosis. But different results in laboratories which happen because of lack of standard methods, are the disadvantages of molecular methods. For solving this issue in this study designing of competitive Internal control(IC) through PCR-cloning has been attempted. Matherial & methods: But using of special primers for IS6110 target, PCR test was optimized. Besides,tho composit primers for IC-SAL were designed thene the PCR was optimized. The IC-SAL which amplified in nonstrigent condition, was ligated in pTZ57R plasmid, transformed in E.coli JM107 and was cloned. Specificity and sensitivity of test wese determind. Results: PCR amplicon for SAL and IC-SAL with special primers were 2845bp and 662bp respectively, so there was a significant different between their size. Sensitivity of PCR for SAL DNA was 12 bacteria and the IC amplified in the ranges between 10-10,000,000 bacteria. Discussion: Despite of high speed and accuracy of PCR, false positive and negative results which are caused because of PCR inhibitors, are the important problems of this technic that can reduce its efficiency. Using another DNA as an internal control can detect these inhibitors. Indeed, amplification of this DNA shows correct amplification and detection steps.

Keywords: salmonella typhi, PCR, Internal control