PCR STANDARDIZATION FOR IDENTIFICATION OF *Escherichia coli* STRAINS PRODUCING SHIGA TOXIN (STEC)

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The toxigenic shiga group comprises 200 Escherichia coli serotypes producing Shiga toxins, or verotoxins, (Stx). This nomenclature is due to its similarity to the toxin produced by *Shigella dysenteriae* type I. The Stx group is classified in Stx two main classes, named immunologically distinct Stx1 and Stx2. Both toxins have variants: Stx1 presents the Stx forms Stx1a, Stx1c and Stxd, while Stx2 are known for displaying forms, STxB, Stxc, Stxd, Stxe, Stxf and Stxg, where an isolated STEC can produce only one toxin, variant or both. These bacteria are recognized as an important group of emerging pathogens and have become a major public health challenge involved in foodborne disease outbreaks and possess a high degree of infectivity in humans, because even when present in low amounts in ingested food (10 CFU), they are capable of causing infection. The traditional identification method is by standard bacteriological tests of Shiga toxin-producing strains, however this is slow and laborious. In this context, the aim of this study was to standardize a polymerase chain reaction to identify *E. coli* producing Shiga toxin. An *Escherichia coli* strain, O157:H7 ATCC 43895, provided by the Fundação Oswaldo Cruz was revitalized in agar eosin and Methylene Blue (EAM), Oxford agar and Rambach agar, and subsequently subjected to DNA extraction with the aid of a commercial Blood and Tissue kit (Qiagen®) and quantification by fluorimetry using Quibit (Invitrogen®). The amplification conditions of a fragment of the 484 bp Slt2 gene by PCR using SLT-IIF primers (5' – GTTTTTCTTCGGTATCCTATTCCG – 3' and SLT-IIR (5' – GATGCATCTCTCTGGTCATTGTATTAC – 3') were established and optimized. DNA strains of reference *Enterobacteriacea* were also used for reaction specificity confirmation. The analytical sensitivity was established by dilution of the target DNA to determine the limit of detection. Each experiment was performed in triplicate. The PCR was standardized using 20 ng target DNA, 10 mM dNTPs, 200 nM of each primer, 2.5 mM $MgCl_2$, 1U Taq DNA polymerase and 59°C annealing. Under these conditions, it was possible to successfully carry out the amplification of the Stx2 gene and identify of potentially producing Shiga toxin (STEC) reference strains. The technique was shown to be quick and efficient, with an analytical specificity of 100% and a limit of detection of 3 ng / DNA of *E. coli* O157: H7.

Keywords: *Escherichia coli* O157:H7, Stx2 gene, Verotoxin (VETC)