INTRODUCTION AND OBJECTIVES: Considered the second world cause of infant mortality, diarrhea is considerate as a neglected disease, especially in prolonged microbiological diagnosis. This disease is caused by a range of pathogens like diarrheagenic *Escherichia coli* (DEC), including the pathotypes enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC), differing from each other by the virulence factors, resulting in different forms of aggression of enterocytes. Taking these together our aim was to develop a reaction of Multiplex Polymerase Chain Reaction (mPCR) to detect DEC, making the most simple and effective diagnosis.

MATERIALS AND METHODS: Using bioinformatics program (MPprimer 2.0), we designed oligonucleotide primers specific for *bfpA* and *escV* (EPEC), *elt* (ETEC), *aaiC* (EAEC), *ipaH* (EIEC) and *stx1* and *stx2* (STEC) pathotypes. Standardization of the method was performed in a thermocycler (Applied Biosystems©) using PCR reagents (Invitrogen©) and total DNA of reference strains for each pathotype. Amplification temperatures were performed between 58°-62°C. RESULTS AND CONCLUSIONS: The reaction was standardized into three distinct systems constituted by the following primers: Reaction 1: *aaiC*, *escV* and *bfpA*; Reaction 2: *stx1* and *ipaH*; Reaction 3: *stx2* and *elt*. PCR products were analyzed in 2.5% agarose gel electrophoresis stained with Syber SAFE (Invitrogen©). The amplification sizes suitable for diagnosis: *aaiC* (183pb), *escV* (266pb), *bfpA* (478pb), *stx1* (130pb), *ipaH* (393pb), *stx2* (346pb) and *elt* (629pb). In conclusion, this mPCR system can be an useful method for the diagnosis of DEC, requiring validate this method with the strains isolated from stools and foods.

ACKNOWLEDGEMENTS: Araucaria Foundation and CAPES.

KEY WORDS: Multiplex PCR, diarrhea, diarrheagenic *Escherichia coli*. 