Peri-implantitis is the main cause of implant failures. Peri-implantitis is provoked by the presence of bacterial infiltration around Implant-Abutment Connection (IAC). Reduction of bacterial leakage may be achieved by improving the accuracy and precision of the two pieces of IAC. The aim of the present in vitro study was to evaluate bacterial microleakage from the inside to the outside of the IAC, testing the efficacy of three new designs of internal conical connection (FN - nano-fix -, NQ - uNiQo - and Elisir implant systems by FMD, Rome, Italy). To identify the efficacy of three new IAC, the passage of genetically modified Escherichia coli across IAC was evaluated. A total of 17 implants were used (5 FN, 6 NQ and 6 Elisir). All implants were immersed in a bacterial culture for 48 h and bacteria amount was then measured inside and outside IAC with Real-time PCR. Bacterial quantification was performed by Real-Time Polymerase Chain Reaction using the absolute quantification with the standard curve method. In all the tested implants, bacteria were found in the inner side, with a median percentage of 1.9% FN, 1.4% NQ and 2.6% Elisir. The analysis revealed that in both cases (internally and externally), bacteria grew in the first 48 hours but subsequently started to die, probably due to nutrient consumption. Of the three, the most efficacious connection was NQ. Within the limitations of this study, it was concluded that the best implant connection reducing bacterial leakage at IAC level was NQ (NQ implant system by FMD, Rome, Italy).