In USA there are biological indicators available where a foam packaging is used to increase their biological resistance. Also self-contained biological indicators (SCBIs) are tiny PCDs containing a BI inside. According to ISO standards, which are historically derived from US standards, the resistance of BIs has to be tested inside of an SCBI.

The challenge in a sterilization process to provide a sterile product however has two different requirements:

1. Penetration challenge
   If instruments have a complex design like arthroscopic instruments with hollow tubes, a sterilization process can only be successful if the sterilization agent is touching the most difficult areas inside of the instrument. Without exchanging the air inside of such tubes against the sterilization agent, no sterilization could be carried out. This challenge is called “penetration challenge”.

2. Kill challenge of a biological indicator
   Biological indicators or pathogenic germs on instruments may have a different resistance and require a shorter or longer sterilization time before all alive spores are inactivated. The definition of the resistance of a biological indicator depends on the spore type with its decimal reduction value (D-value) and the number of spores, called “$F_{Bio}$-value” and is defined:

**Resistance = $F_{Bio}$ = log pop x D-value**

spore amount — depending on the spore type

Very often both challenges are not differentiated but the term “sterilization challenge” is quite often used, e.g. when the resistance of self-contained biological indicators (SCBIs) is tested, the standard requires testing the resistance of the BI inside the SCBI. However, the SCBI has the penetration resistance and a kill challenge resistance in addition. The kill challenge is important to know how long a sterilization process is carried out with its $F_0$-value, the penetration challenge in addition has to be independently checked, how good the air removal and sterilization agent penetration is carried out which is purely sterilizer process dependent.

Therefore, when the resistance of an SCBI is tested, the BI has to be taken out of the SCBI and tested separately to check the kill challenge (D-value).