Making Internal Amplification Control Mandatory for Diagnostic PCR

The explosive increase since the beginning of 1990s in the number of publications reporting PCR-based methods for detection or molecular typing of food-borne pathogens has attracted the attention of end user laboratories.

However, the well-recognized difficulties in reproducing published tests due to variation in the performance of PCR thermal cyclers (7) and in efficiencies of different DNA polymerases and to the presence of PCR inhibitors in the sample matrix have hampered implementation in end user laboratories. This particularly applies to laboratories with quality assurance programs.

It is necessary to have PCR-based guidelines available as internationally recognized standards (5). Currently, the lack of international standards often forces end user laboratories to spend substantial resources on adaptation of the published tests. Although many commercial PCR kits are available, it is important that end users and reference laboratories have access to open-formula, noncommercial, and nonproprietary PCRs in which the information on target gene and reagents is fully available.

The prerequisites for a PCR published in the scientific literature to be adopted as a standard are that it has to be nonproprietary and that it has to have been validated through a multicenter collaborative trial according to the international criteria (1, 2, 5). Multicenter trial validation of noncommercial PCRs for detection of zoonotic pathogens has been performed by a European validation and standardization project (FOOD-PCR [http://www.pcr.dk]) involving 35 laboratories from 21 countries (4, 6).

A major drawback of most published PCRs, surprisingly even to date, is that they do not contain an internal amplification control (IAC). An IAC is a nontarget DNA sequence present in the same sample reaction tube which is coamplified simultaneously with the target sequence. In a PCR without an IAC, a negative response (no band or signal) can mean that there was no target sequence present in the reaction. But it could also mean that the reaction was inhibited due to malfunction of the thermal cycler, incorrect PCR mixture, poor polymerase activity, and, not least, the presence of inhibitory substances in the sample matrix. Conversely, in a PCR with an IAC, a control signal will always be produced when there is no target sequence present. When neither IAC signal nor target signal is produced, the PCR has failed. Thus, when a PCR-based method is used in routine analysis, an IAC, if the concentration is adjusted correctly, will indicate false-negative results. It is the false-negative results that turn a risk into a threat for the population, whereas a false-positive result merely leads to a clarification of the presumptive results by retesting the sample.

The European Standardization Committee, in collaboration with International Standard Organization, has proposed a general guideline for PCR testing that requires the presence of IAC in the reaction mixture (3). Therefore, only IAC-containing PCRs may undergo multicenter collaborative trial, which is a prerequisite for standardization.

The scientific journals must provide the source of new PCR-based methods suitable for standardization. Therefore, we propose that henceforward the editorial boards of applied microbiology journals require inclusion of an IAC in diagnostic PCR reported in submitted manuscripts. This could be done by providing a specific section devoted to PCR in their instructions to authors.

REFERENCES

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