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Metrology in Thermal cyclers: a new approach

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Abstract. Metrological laboratories propose different protocols to qualify thermal cyclers, each depending on a different approach. But all these protocols may not match the laboratory need. Some questions must be answered before any metrological test. Has the qualification protocol be performed with a fixed temperature program? What must be done if the metrological qualification is out of specification? Can specifications be set already?
With reference to standardised publications [1-3], we are going to try to focus on the current state of art about metrological qualification. It seems to be necessary to confirm any metrological test by a biological one. And to conclude that a difference between measured value and fixed value does not necessary lead to an end-of-life your thermal cycler even a false genetic analysis.

Introduction

Biological or pathological laboratories may have the opportunity to conduct genetic analyses to improve patient's diagnostic. Among them may be conducted: structural chromosomes study, expression profile and test for cancer prognosis, … Specific DNA or RNA sequences are amplified to create thousands to millions of copies; then, the focused segment of DNA/RNA can be studied. A common and powerful technology to achieve these high specific sequences, is called PCR (Polymerase Chain Reaction). This technology relies on a thermal cycling program with three steps: DNA/RNA denaturation, annealing and elongation. Many factors can influence the reaction yield and among them, temperature is critical due to enzymatical reactions.

Technical aspects of PCR

PCR is mainly constituted of 3 steps: denaturation; annealing and elongation. But Pre-PCR steps may have an impact on the final result (f.i. reverse transcription). Each of the PCR steps have they own robustness. Denaturation is a very robust step, but a too high temperature may have a nasty effect on the Taq polymerase. Annealing seems to be the more critical step for PCR yield and specificity [4]. Elongation is dependent on the polymerase activity range. Thermal cyclers are used to automatized these three steps. As all measurement apparatus, temperature and times are quantities to be qualified with metrological protocols which ensure quantities to be relied on the International System.

Potential variability factors

Thermal cyclers are the result of a quite complex technology, as shown in Figure 1, so that comply it against specification (maximum permissible measurement error) is not so easy.

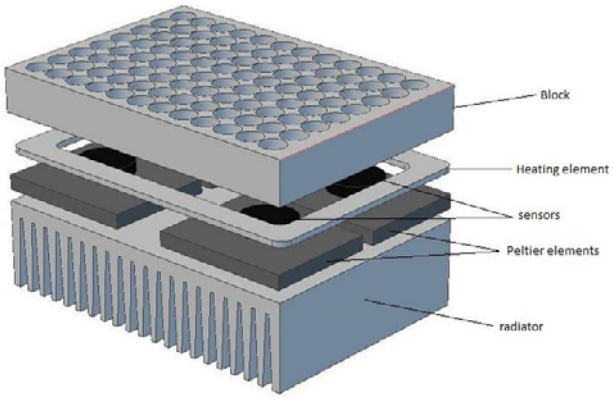

Fig. 1. Detail of thermal cycler generating heat system

There is a great variability into thermal cyclers brand to brand, model to model but also into the same model of instrument, as shown in Figure 2 [5-6]. A lot of factors may influence the temperature profile: The use frequency; environmental conditions; quality of integrated elements (Peltiers elements; temperature sensors …)

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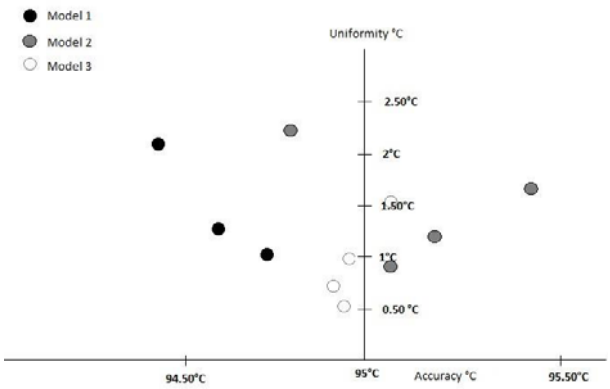

Fig.2. Uniformity versus accuracy in different thermal cyclers

Metrology in a thermal cycler

In order to rule on the conformity of thermal cyclers, it is necessary to control several temperature parameters on the instrument. Several factors may have an influence on the PCR result:

Uniformity is one of the most critical parameter. Instrument uniformity is defined as the difference between the hottest spot and the coldest one for each controlled temperature. This parameter is important to be sure that all samples are treated in a similar way.

Overshoot / Undershoot: Overshoot and undershoot are due to the block inertia, after heating or cooling ramps, the metallic block need to be stabilized at the desired temperature. This factor is depending on the thermal cycler regulation and we will see later that this may have a great impact on the PCR quality.

Accuracy: This parameter is defined by the ability of the instrument to give the desired temperature. The difference between the measured average temperature and the requested temperature is the accuracy deviation.

Other parameters such as ramp rates are good indicators for instrument wornness. The temperature control of the heated lid is also useful to get a full overview of the instrument health.

As shown in Figure 3, uniformity and accuracy are then compared to specifications, if they have been defined by the laboratory, to fix with the metrological conformity of thermal cyclers.

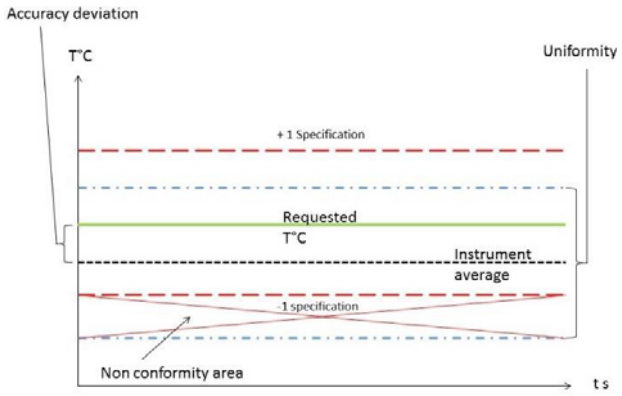

Fig. 3. Conformity area in a metrological qualification

Biological impact of a metrological non-conformity

The most important deviation in a thermal cycler is the overshoot at beginning of denaturation. It may affect the polymerase efficiency. Possible consequences may be loss of yield and elevation of the crossing point. In case of very high and long overshoot, no detectable amplification may occur.

Undershoots at beginning of annealing may induce non specific amplification products. As for overshoots, height and duration have to be considered, to identify risks.

Uniformity is also a critical point. As denaturation is a robust step, it has mainly a strong influence on the annealing steps. A good uniformity insures you that all your samples or PCR points are treated in the same way. It is necessary to be sure that no points of the PCR block will lead you to non specific products; no loss of yield or no polymerase inactivation.

Accuracy is important to be sure that your instrument delivers a correct average temperature at each PCR steps.

A biological test to confirm

In order to confirm instrument conformity, it is recommended to test (when possible) the most deviating points of the block to verify that the PCR is not affected by the thermal cycler temperature profile. Two different cases: The laboratory has defined specifications that can be compared to the instrument temperature profile, but as it has been described before, the conformity depends on several factors. The laboratory did not define specifications; in that case, the conformity can be verified by testing positive controls in the hottest and the coldest points of the block, these points should be defined during the instrument metrology. In real time PCR, it is recommended to test positive controls at 3 times the detection limit. In this case, the crossing points have to be compared to the expected value. This gives a good overview of PCR yield in the worth points of the instrument.

Conclusion

A metrological decision (conformity versus non-conformity) of a thermal cycler seem to be not sufficient to conclude if a PCR reaction can achieve the expected result. As each PCR reaction has its own requirements (different temperature programs), it may be necessary to dedicated a thermal cycler with poor performance with a less specific PCR reaction.
Both metrology and biology must coexist to qualify thermal cyclers, and master the analysis.

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