

Thermal cycler temperature accuracy

A comparison of several models

Introduction

Thermal cycler temperature accuracy may be a critical factor in the success or failure of a PCR reaction. Temperature accuracy is particularly important during annealing temperature optimization, which requires both accuracy and consistency in the thermal cycler block. This study compares the accuracy of thermal cycler blocks by taking measurements using precise temperature verification equipment. Temperature accuracy is measured both in isothermal mode, where the entire block is at the same temperature, and in Applied Biosystems™ VeriFlex™ mode or gradient mode, where different sections

of the thermal block are set to different temperatures. We compare several currently available thermal cyclers against each other and also against the manufacturers' published specifications.

Materials and methods

The instruments tested in this study are shown in Table 1. The same equipment and methods were used to test all of the thermal cyclers, and those methods are described in the following sections.

Table 1. Instruments tested in this study.

Manufacturer	Model name	Cat. No.
Bio-Rad	C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module	185-1196
Bio-Rad	T100 Thermal Cycler	186-1096
Bioer	Life ECO Thermal Cycler	BYQ6078
Eppendorf	Mastercycler X50a	6313 000.018
Takara	Dice Touch	TP350
Thermo Fisher Scientific	ProFlex 96-Well PCR System	4484075
Thermo Fisher Scientific	VeritiPro 96-Well Thermal Cycler	A48141
Thermo Fisher Scientific	SimpliAmp Thermal Cycler	A24811
Biometra	TAdvanced	846-x-070-201
Biometra	TOne	846-x-070-301

Measurement of block accuracy in isothermal mode

Block accuracy was measured using the Applied Biosystems™ VeriFlex™ 96-Well Temperature Verification Kit (Cat. No. 4377669) with 3 temperature probes. Calibration standards are traceable to the US National Institute of Standards and Technology (NIST). Each probe measures temperature at 8 positions, providing a total of 24 temperature measurements at various points on the block. A diagram of the temperature probe layout is shown below.

Block temperature accuracy was determined by taking the average of these 24 data points and subtracting that value from the set point programmed into the instrument. Each accuracy measurement was taken after the instrument had been holding the temperature for 180 seconds. This procedure was repeated 3 times for each of the following temperature set points: 45, 55, 72, 85, and 95°C. The accuracy data for each instrument were then compared to the published specifications found on each manufacturer’s website.

Measurement of VeriFlex Blocks or gradient block accuracy

Each instrument in this study was tested using Applied Biosystems™ VeriFlex™ Blocks or gradient block technology to vary temperatures. The Applied Biosystems™ ProFlex™, VeritiPro™, and SimpliAmp™ thermal cyclers are equipped with VeriFlex Blocks, which are independently controlled temperature blocks. All of the other thermal cyclers tested use only a single thermal block. A commonly used annealing temperature of 60°C was used as the approximate center point, and then each block was tested using the maximum temperature range allowed by the thermal cycler. For example, a thermal cycler with a maximum temperature range of 20°C was tested at the range shown in Table 2.

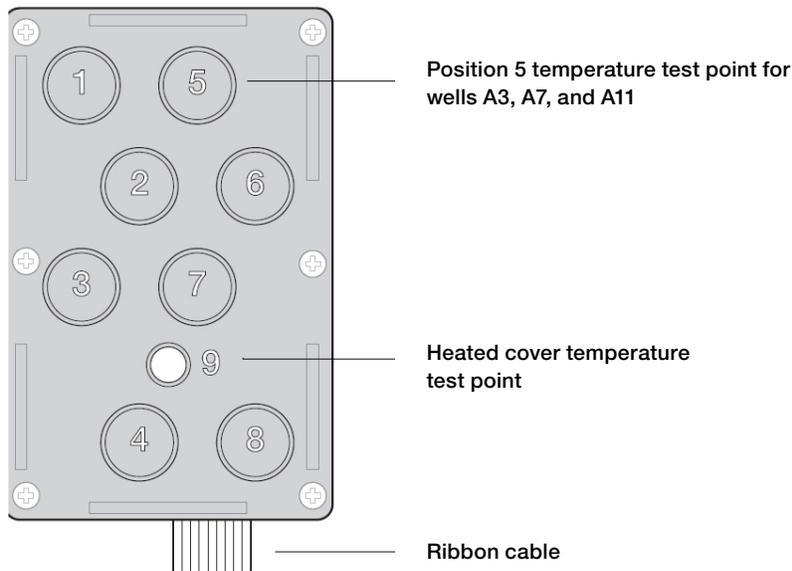


Table 2. Sample data for temperature range measurement.

Temperature set point* (°C)	50.5	51.7	53.6	56.2	58.8	61.2	63.8	66.4	68.3	69.5
Block temperature measurement† (°C)	50.6	51.8	53.7	56.1	58.7	61.2	63.8	66.3	68.4	69.4
Delta‡ (°C)	0.1	0.1	0.1	-0.1	-0.1	0.0	0.0	-0.1	0.1	-0.1

* As displayed on the interface of the machine.

† As measured using the VeriFlex 96-Well Temperature Verification Kit.

‡ In the Results section, the largest delta figure observed across the whole temperature range is reported.

Results

Table 3 shows temperature accuracy measurements in isothermal mode, where the entire block is at the same temperature. Table 4 shows temperature accuracy measurements in VeriFlex mode or gradient mode, where different sections of the thermal block are set

to different temperatures. Published values of the gradient temperature range for each thermal cycler, as well as the number of different temperatures measured in that range, are also shown (Table 4).

Table 3. Temperature accuracy measurements in isothermal mode.

Thermal cycler	Published specification	Set point				
		45°C	55°C	72°C	85°C	95°C
Bio-Rad C1000 Touch Thermal Cycler	±0.2°C at 90°C	0.18	-0.03	-0.17	-0.21	-0.20
Bio-Rad T100 Thermal Cycler	±0.5°C of programmed target	0.21	0.24	0.17	0.06	-0.03
Bioer Life ECO Thermal Cycler	±0.1°C (55°C); ±0.2°C (90°C)	0.20	0.03	-0.26	-0.29	-0.29
Eppendorf Mastercycler X50a	±0.15°C	0.13	0.06	-0.04	-0.10	-0.16
Takara Dice Touch	±0.5°C (30–99°C)	-0.09	-0.15	-0.24	-0.29	-0.33
ProFlex 96-Well PCR System	±0.25°C (35–99.9°C)	-0.07	-0.04	-0.02	0.00	0.02
VeritiPro 96-Well Thermal Cycler	±0.25°C (35–99.9°C)	0.00	0.06	0.12	0.08	-0.01
SimpliAmp Thermal Cycler	±0.25°C (35–99.9°C)	0.01	0.02	0.05	0.10	0.14
Biometra TAdvanced	±0.1°C	0.01	0.04	0.10	0.18	0.24
Biometra TOne	±0.1°C	0.11	0.16	0.24	0.33	0.39

Table 4. Temperature accuracy measurements in VeriFlex mode or gradient mode.

Thermal cycler	Maximum VeriFlex mode or gradient mode temperature range	Number of different temperatures in VeriFlex mode or gradient mode	Accuracy (maximum discrepancy of set point compared to actual measurement)
Bio-Rad C1000 Touch Thermal Cycler	24°C	8	±1.2°C at 75°C
Bio-Rad T100 Thermal Cycler	25°C	6	±0.6°C at 50°C
Bioer Life ECO Thermal Cycler	30°C	12	±0.1°C at 55°C
Eppendorf Mastercycler X50a	30°C	12	±0.15°C at 55°C
Takara Dice Touch	24°C	12	±0.8°C at 58.9°C
ProFlex 96-Well PCR System	25°C	6	±0.2°C at 75°C
VeritiPro 96-Well Thermal Cycler	30°C	6	±0.25°C at 35–99.9°C
SimpliAmp Thermal Cycler	20°C	3	0.0°C at 60°C
Biometra TAdvanced	30°C	6	±0.20°C at 55°C
Biometra TOne	20°C	12	±0.20°C at 55°C

Discussion

Temperature accuracy specifications vary across instruments found in the market. Here, thermal cycler temperature performance is compared side by side, measured using the same system and in the same way. Temperature accuracy varied across the models tested, compared with published specifications.

Gradient block accuracy is not a specification that is typically published, even though it could be of critical importance. Often, VeriFlex Blocks or gradient blocks are used to optimize primer annealing temperature.

If the temperature set point of an instrument does not correspond to the actual temperature of the block, further temperature optimization could be required. The temperature accuracy data from thermal cyclers in gradient mode clearly show some discrepancies between measured temperatures and the temperatures displayed on the instrument interface.

For best results, we recommend using a thermal cycler that has high temperature accuracy in both isothermal mode and gradient mode.

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