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The use of infrared thermography as a novel approach for real-time validation of PCR thermocyclers

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Running head: Thermocycler validation by thermography

Abstract

Validation of PCR thermocycler performance is crucial to obtain reliable results. In this study, infrared (IR) thermography was evaluated as a novel validation tool. After stabilisation, no significant difference in the temperatures recorded using thermography and a reference block-based system was found. By employing IR thermography information about the length of the time until temperature stabilisation in the sample could be obtained. This study shows the potential of using IR thermography for validation of thermocyclers.

Keywords: PCR, thermocycler, thermal cycler, infrared thermography, validation

Accurate thermocyclers is a prerequisite for all PCR based applications including diagnostic PCR for foodborne pathogens. Differences in temperature performance, between brands and types of cyclers has been demonstrated to influence PCR performance (Kim et al. 2008; Saunders et al. 2001; Yang et al. 2005). Proper validation of the thermocycler performance is one factor limiting the spread of diagnostic PCR to end-users (Malorny et al. 2003). Validation is important to ensure proper and reproducible results from PCR analysis and when transferring an assay between different thermocycler models (Schoder et al. 2005). It can also reveal problems caused by poor thermocycler performance (Kim et al. 2008; Schoder et al. 2005).

Ideally, a validation procedure should be able to measure the temperature in-sample simultaneously in all wells. Two main approaches has been proposed in the ISO/TS 20836 standard (Anonymous 2005): biochemical and physical performance tests. A biochemical procedure may consist of a temperature sensitive non-robust PCR method (Anonymous 2005; Yang et al. 2005). However, when experiencing problems it can be hard to identify the source of the problem and therefore it is feasible to supplement the biochemical validation procedure with a physical one where the actual temperature within each well during temperature cycling is measured (Anonymous 2005; Schoder et al. 2003; Schoder et al. 2005; Yang et al. 2005). Probes for measuring temperatures in-tube have been developed (Schoder et al. 2003; Yang et al. 2005). Alternatively, the temperature distribution in the heating block (not in the sample) can be recorded using temperature sensitive probes. Several commercial systems are available for this purpose (e.g. TAS, Quanta Biotech, Somerset, UK and DRIFTCON, GENO-tronics, Landgraaf, The Netherlands). However, heat transfer and distribution through the sample itself, not the block alone, is crucial. Substantial variation in the effective length of a temperature step after the sample has reached the set temperature has been reported (Kim et al.

2008). For sensitive assays, this might affect the outcome of PCR and there is a need to calibrate the block temperature with the performance of PCR.

Infrared (IR) thermography is a technology to detect and visualize IR energy radiating from an object for displaying images of temperature measurement or temperature distribution (for a review see, (Meola and Carlomagno 2004). The equipment used to perform IR thermography is often referred to as an IR camera or thermocamera. IR thermography has been employed as a tool for non-destructive evaluation of a vast variety of materials and components (Meola and Carlomagno 2004; Purohit et al. 2006). The objective of this study was to demonstrate that IR thermography can be applied as an alternative non-destructive in-tube method for physical validation of thermocycler performance.

In this study, the DRIFTCON system 96V-15 was employed as a reference method (referred to as the probe-based system). This system consists of a so-called "probe fixture", a construction at which 15 thermistors are fixated in a holder, and placed in the block as a whole, prior to the measurement. A temperature program (30°C for 60 s., 94°C for 180 s., 65°C for 180 s., 72°C for 180 s. and 30°C for 60 s.) was run on a DNA Engine PTC-200 thermocycler (MJ Research, Waltham, MA). The probe-based system was set-up according to the manufacturer's instructions (Fig. 1) using software v. 4.5.0 and analysis software v. 1.1.3.0. Examination of the thermocycler was done without the lid covering the PCR plate. For the thermographical evaluation, all wells (Fig. 1) of a standard 96-well PCR plate was filled with 50 µl of double distilled water and overlaid with a drop of mineral oil (Sigma-Aldrich, Brøndby, Denmark). Two thin strings of steel wire were used to sure PCR plate to the heating block. A thermocamera (AVIO Advanced Thermo TVS-500, Nippon Avionics, Tokyo, Japan) was placed above the heat block, pointing directly down at the

thermocycler; at a distance of approximately 75 cm. Detailed analysis of heat distribution and time dependent heat spread in an individual sample was done using the GORATEC thermography studio 2007 software (GORATEC Engineering, Neufraunhofen, Germany). Both systems were calibrated in accordance with the law concerning legal metrology, which documents traceability to national standards (Anonymous 2004). The performances of the two methods were compared including parameters such as well-to-well differences and time until temperature stabilisation (Tables 1 & 2, Fig. 2). T-test was used to see if there were any significant differences in temperatures obtained for the two systems using Microsoft Office Excel 2007 (Microsoft).

Generally, both the thermocamera and the probe-based system were appropriate to use for validation of thermocycler performance. However, the properties of the systems differ (Table 1). The probe-based system measures the temperature in 15 wells simultaneously using temperature sensitive probes that are in direct contact with the heating block walls (Fig. 1). This system is specialized to record the heat distribution equipment such as a PCR heating block and has dedicated software for this purpose. However, it cannot provide information on the mechanisms and rate of heat transfer to and from the PCR samples. It is only possible to register this information by in-tube physical validation, but since no such systems are available commercially, the practical use of this approach is limited. The thermocamera has the ability to survey all wells simultaneously and obtain two- and three-dimensional (Fig. 3) temperature profiles. However, the software is difficult to manage and not dedicated to validation purposes. Even though the temperature accuracy of the thermocamera is not as high as for the probe-based system (Table 1), the well-to-well variances can be displayed using thermography and wells not performing optimal can be singled out.

The performance of the tested thermocycler was recorded and compared to manufacturer's specifications (Tables 1 & 2). According to these specifications the temperature accuracy of the thermocycler should be ±0.3ºC of programmed target at 90ºC and the temperature uniformity ±0.4ºC well-to-well within 30 s of arrival at 90ºC. The temperature uniformity specification was not met at the close temperature of 94ºC, where the temperature measured was 93.64ºC and the spread 0.80ºC after 30 s. It is therefore likely that the thermocycler does not perform according to specifications and it should therefore be subjected to service and calibrations as first attempt to regain the correct performance. The thermocycler employed in this investigation was an older model and was not calibrated. Calibration has previously been noted as an important factor when assessing the performance of thermocyclers (Saunders et al. 2001). Nonetheless, to consider the usefulness of thermography as an alternative method of validation of thermocyclers the performance of the cycler is not of major importance as long as reproducible results are obtained.

The time registered by the thermocamera for the sample to reach target temperature was considerable longer compared with the heating block (Table 2, Fig 2); the sample did not reach the target temperature until at the end of each temperature hold. This is not surprising, as heat transfer is slower in a 50-µl sample compared with the heat block. The use of mineral oil to avoid evaporation instead of the heated lid might also have affected the length of the time to reach target temperature. However, employing thermography allowed us to obtain information about the length of this lag time. It is a crucial source of error and will have an influence on the quality of the PCR product if not taken into account when a PCR protocol is programmed especially when transferring an assay between different PCR cyclers (Kim et al. 2008). Once the temperatures in the samples were stable, there was no significant difference between the average well temperature measured with the probe-based system or the thermocamera (Table 2, Fig 2).

In conclusion, it was observed using a model thermocycler, that IR thermography has the possibility to be applied as an alternative way to obtain in-tube temperature measurements during thermal cycling to form the basis for validation of thermocycler performance. Further studies are needed on other thermocycler models to confirm this observation and assure the reproducibility of the system.

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Table 1 Comparison of the performances of the probe-based system and the thermocamera for validation of PCR thermocyclers.

Parameter	**Equipment**[a]	
	Probe-based	**Thermocamera**
Accuracy [b]	± 0.15 ºC	± 2 ºC
No of temp measurement points	15[c]	5[d]
Measurement data range[b]	10-105 ºC	-40- ~500 ºC[e]
NIST traceability[b, f]	Yes	Yes

[a] Probe-based system: DRIFTCON 96V-15, GENO-tronics; Thermocamera: AVIO Advanced Thermo TVS-500, Nippon Avionics

[b] Performance parameters as given in manufacturers specifications

[c] Performance parameters given according to the layout of this study. Other versions of the system available with more probes

[d] Performance parameters given according to the layout of this study. Five points can be analysed simultaneously during thermocycling, with the possibility of analysing more points in the software after the end of the experiment.

[e] Filters available to extend temperature range

[f] NIST: National Institute for Standards & Technology. National measurement laboratory (Washington DC, USA).

Table 2 Comparison of thermocycler performance using the probe-based system measuring in the block and the thermocamera measuring in-sample.

Measurement point	Parameters measured[a]	T_{start} (ºC)→ T_{target} (ºC)					
		30→94		94→65		65→72	
		Probe based	Thermo camera	Probe-based	Thermo camera	Probe based	Thermo camera
After 15 s[b]	T (ºC)	93.6	54.8	64.7	80.8	71.6	70.1
	ΔT_{well}	0.8	4.1	0.6	5.6	0.4	1.1
After temperature stabilisation[c]	T (ºC)	93.6	94.0	64.8	64.9	71.8	71.6
	ΔT_{well}	0.8	1.7	0.4	0.9	0.5	0.9
	t_{stab} (s)	41	143	25	148	8	92

[a] T : Average temperature in the measured wells; ΔT_{well}: Temperature un-uniformity in the sample block, calculated out of the highest temperature and the lowest temperature in the sample block (defined as spread in the Driftcon system); t_{stab} : time from the beginning of each step as defined by the probe based system until temperature stabilisation defined as the time for temperature to be within ± 0.3ºC of the temperature at the end of each temperature plateau.

[b] 15 s after the beginning of each step as defined by the probe based system.

[c] At the end of each temperature plateau.

Legends to figures:

Figure 1

Positions (wells) of PCR plate were measurements were made using the probe base system (P) and the thermocamera (T).

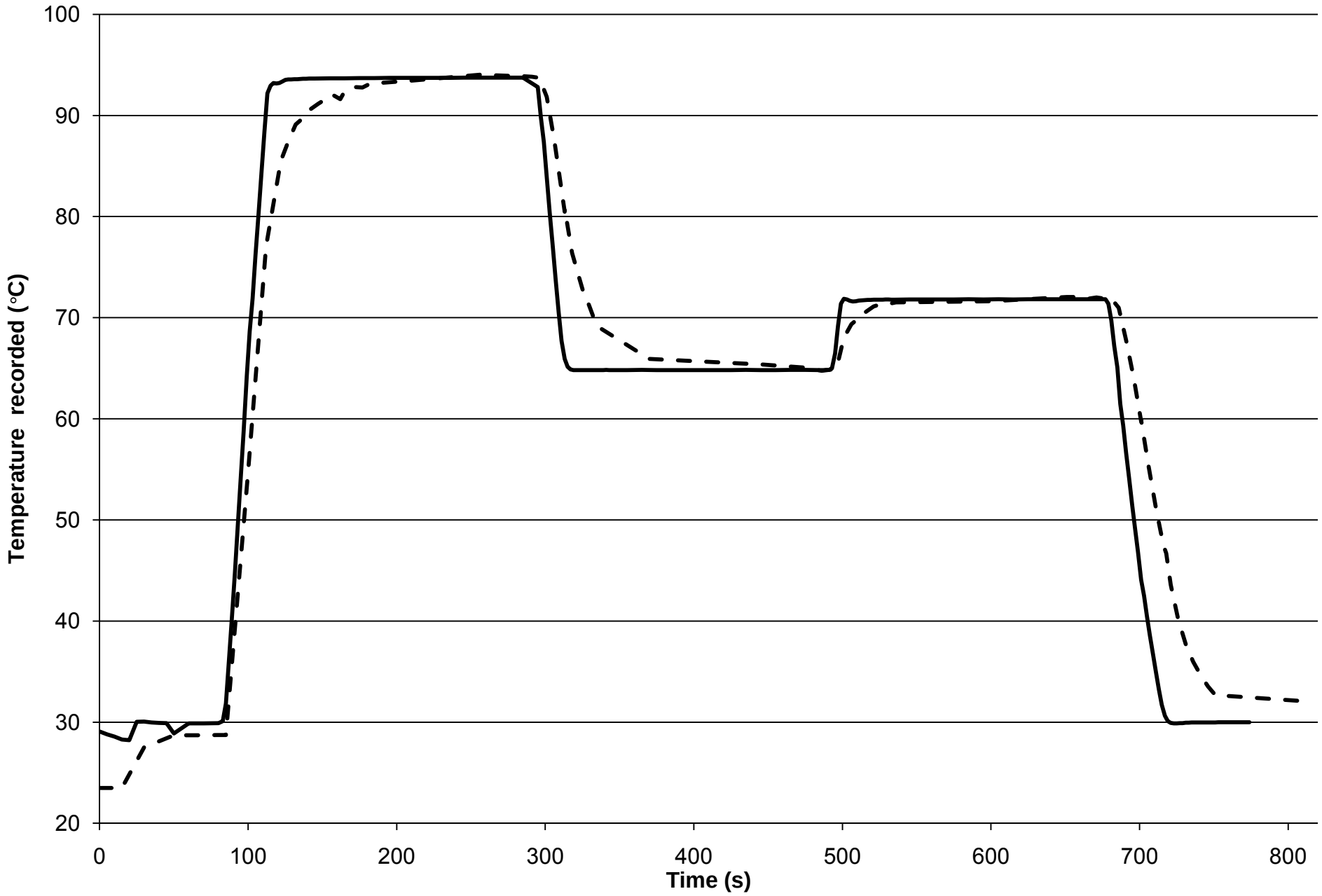
Figure 2

Recording of the temperatures using the probe based system (solid line) in the block and the thermocamera (dotted line) in the sample.

Figure 3

The 3D image of the individual wells in a PCR plate recorded by the thermocamera. The height of the single cones represents the temperature at the liquid surface of the wells; the higher the cone, the hotter the sample.

	1	2	3	4	5	6	7	8	9	10	11	12
A	P/T			P			P			P		P
B												
C												
D	P						P					P/T
E				P/T						P/T		
F												
G												
H	P			P			P/T			P		P


100
90
80
70
60
50
40
30
20
Temperature recorded (°C)
0
100
200
300
400
500
600
700
800
Time (s)