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APPLICATION NOTE No. 466

“Through space & time”: Eppendorf twin.tec® PCR Plates provide utmost assay reproducibility - implications for NGS workflows.

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Abstract

Constantly increasing complexity and sensitivity of modern life science and diagnostic methods requires that results are highly reproducible throughout several experimental replications often carried in various laboratories and over longer periods of time. A frequently-overlooked influence on the reproducibility, performance and quality of experimental results are laboratory consumables.

Among the several complex methods NGS as technology has matured and emerges as a driver for modern precision medicine, clinical diagnostics and related research studies. With the advent of NGS, reproducibility of the NGS library preparation, often including a PCR step, turns out to be essential and comes to the fore. PCR microtiter plate consumables, which are an integral part of those upstream steps, may substantially vary in their quality and can have a considerably negative impact on the result of the final NGS analysis.

In this application note, by using a qPCR as a surrogate method for amplification driven protocols such as NGS, we demonstrate that Eppendorf twin.tec® PCR plates offer highly reproducible assay performance with a coefficient of variation 0.40%. This performance is independent regardless of whether different wells of the same plate (intra-plate), different plates of the same production lot (intra-lot), or plates of different lots (inter-lot) are compared. Even plates taken from a 4 years old production lot transported to Australia and back to Germany provided the same consistent performance – a demonstration of the high-quality material and high-precision production processes characterizing the Eppendorf twin.tec Plates!

Introduction

The maturing of the NGS technology has spurred a plethora of research and application fields not only in the mainstream life science and diagnostics, but also in fields such as heritage analysis, life style, nutritional advice and forensics. Commonly, these workflows essentially rely on high assay reproducibility: low error rates and large sample throughput are either required to establish the basis for the underlying bioinformatical models or are compared and referenced over long time.
Consumables, such as PCR tubes or PCR plates, may have a considerable influence on experimental results and their reproducibility. Especially PCR plates used in high-throughput workflows may have a dramatic impact in complex analysis systems. Apart from commonly known parameters such as high chemical and biological purity, there are other less known characteristics, which may adversely influence good assay performance of a PCR plate. These include leachables (chemicals released form the plastic), inhomogeneous wall thickness of the tubes or plate wells and also the surface structure or polymer material composition.
To demonstrate the consistency of the Eppendorf twin.tec PCR Plates’ performance we resorted in this application note to a qPCR approach as a surrogate method for NGS (or similar sensitive methods) and evaluated following assay reproducibility parameters:

1) Intra-plate analysis: identical samples analyzed simultaneously in the same plate
2) Inter-plate/intra-lot analysis: identical samples analyzed in different plates within one lot
3) Inter-lot analysis: identical samples analyzed in different plates taken from lots produced in different production years
4) "Space and time analysis": identical samples analyzed in different plates taken from lots, which were shipped around the globe in order to assess if environmental change and time have an effect on the performance of consumables.

To comprehensively address these questions five different production lots were evaluated: currently produced plates, samples of newest product variant (twin.tec Trace PCR Plates) as well as plates, which have travelled the world via sea freight to Australia, were stored and shipped back via air freight in a total journey of 4 years (Fig. 1).
During its journey, including transport and storage phases, the lot was subject not only to time ("aging effect") but also to various environmental influences such as temperature fluctuations or variations in atmospheric pressure.

Figure 1: The "world travel" of one of the lots of Eppendorf twin.tec PCR Plates analyzed in this application note. The lot was transported, stored and „experienced" diverse environmental conditions such as temperature fluctuations or variations in atmospheric pressure before coming back home.

Materials and Methods

Materials

The following Eppendorf twin.tec® PCR Plates 96 skirted (order #0030 128.672/ 0030 128.648) were evaluated:

> Lot 1: K196981L: production mid 2021
> Lot 2: K198752O: production end 2021
> Lot 3: new product variant – twin.tec® Trace PCR Plate 96, samples produced end 2021
> Lot 4: new product variant – twin.tec® Trace PCR Plate 96, samples produced end 2021
> Lot 5: H177163I: production in 2018, traveling from Germany to Australia and back

Methods

Three different plates from each lot were evaluated in a comparative qPCR experiment. The qPCR runs were processed on the same real-time PCR system: CFX96 touch™ (Bio-Rad®) using a qPCR assay detecting the bacteria *Acinetobacter baumannii*.
Each reaction was carried out in a total volume of 20 µL containing 10 µL of Takyon™ No ROX SYBR® 2X MasterMix Blue dTTP (Eurogentec), 0.25 µL of dUTP/UNG Additive for dTTP Kit Conversion (Eurogentec), 0.3 µL of each A. baumannii forward and reverse primers at 20 µM, 4.2 µL nuclease-free water and 5 µL A. baumannii gDNA. The same batches of reagents were used for all experiments and the pipetting steps to prepare the Master mix were performed using electronic Eppendorf Xplorer® plus pipettes.

The Master mix was pipetted into 24 wells of the plate using the electronic Multipette E3x® pipette. The plates were sealed with the Masterclear® real-time PCR Film self-adhesive and centrifuged for 1 min at 500 x g (Centrifuge 5920 R with Rotor S-4x1000). The mix was subjected to the following thermal conditions: 2 minutes at 50°C, 3 minutes at 95°C, followed by 40 cycles of 95°C for 10 seconds and 60°C for 60 seconds. The selected fluorophore was SYBR (Ex 450-490 / Detection 515-530). The Cq-values and amplification data were analyzed using the CFX Maestro™ software from (Bio-Rad). Cq values were determined with PCR Base Line Subtracted Curve Fit and automatic threshold analysis method of the software.

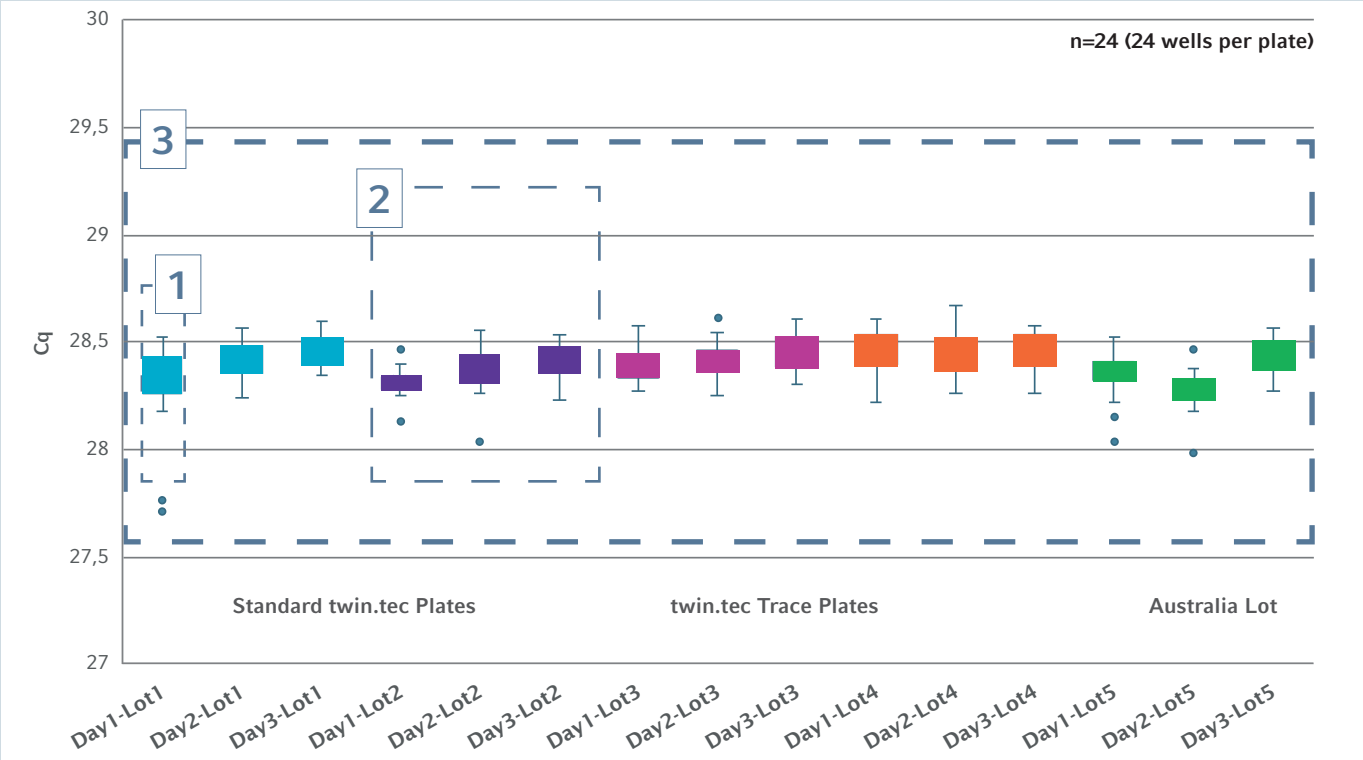
Results and Discussion

The results in figure 2 show that the reproducibility of the intra-plate, inter-plate and inter-lot in the qPCR assay using twin.tec PCR plates is very consistent.

Simply put: independent of which well, which plate and which Lot is selected - the deviation of the obtained Cq values ranges in a narrow window below 0.5 cycles.
It must be kept in mind that even with careful setup, the Cq values are also affected by the other factors of the assay (i.e. day, fluctuations in reagent concentrations, cycling temperature, ...).

Global inter-lot repeatability values were: Cq mean of 28.40 with SD of 0.11 and CV of 0.40. Also, the values of the intra-plate Cq standard deviation (SD) were low and ranged from 0.06 to 0.10 corresponding to the coefficient of variation of 0.23% to 0.39%, respectively.

Scientists commonly use reference samples on each single plate to account for technical variations. Oftentimes those references are pipetted in one column. Figure 3 shows that well position across the plate has no influence on the obtained Cq values. The average signal obtained in three different columns of the plates are very close to each other. (figure 4). The assessed values were close to the ones obtained for the intra-plate analysis. The Cq standard deviation (SD) of the qPCR results obtained across 3 plates per each of the single Lots ranged from 0.08 to 0.14 corresponding to a coefficient of variation of 0.30% to 0.51% respectively. The coefficient of variation is 0.40% demonstrating also that the reproducibility between lots is very high.



1: Intra-plate reproducibility 2: Inter-plate/intra-lot reproducibility 3:Inter-lot reproducibility

Figure 2: Box-plot of intra-plate, inter-plate and inter-lot reproducibility. Each box represents the middle 50% scores of the data. The median Cq is shown by the line within each box and the upper and lower whiskers represent scores outside the middle 50%. Five different lots were analyzed and per each lot 3 plates with 24 wells each were assessed. Dotted rectangles depict various aspects of assay reproducibility: 1: Intra-plate reproducibility, 2: Inter-plate/intra-lot reproducibility and 3: Inter-lot reproducibility. Outlier values are depicted by single dots below or above box plots.

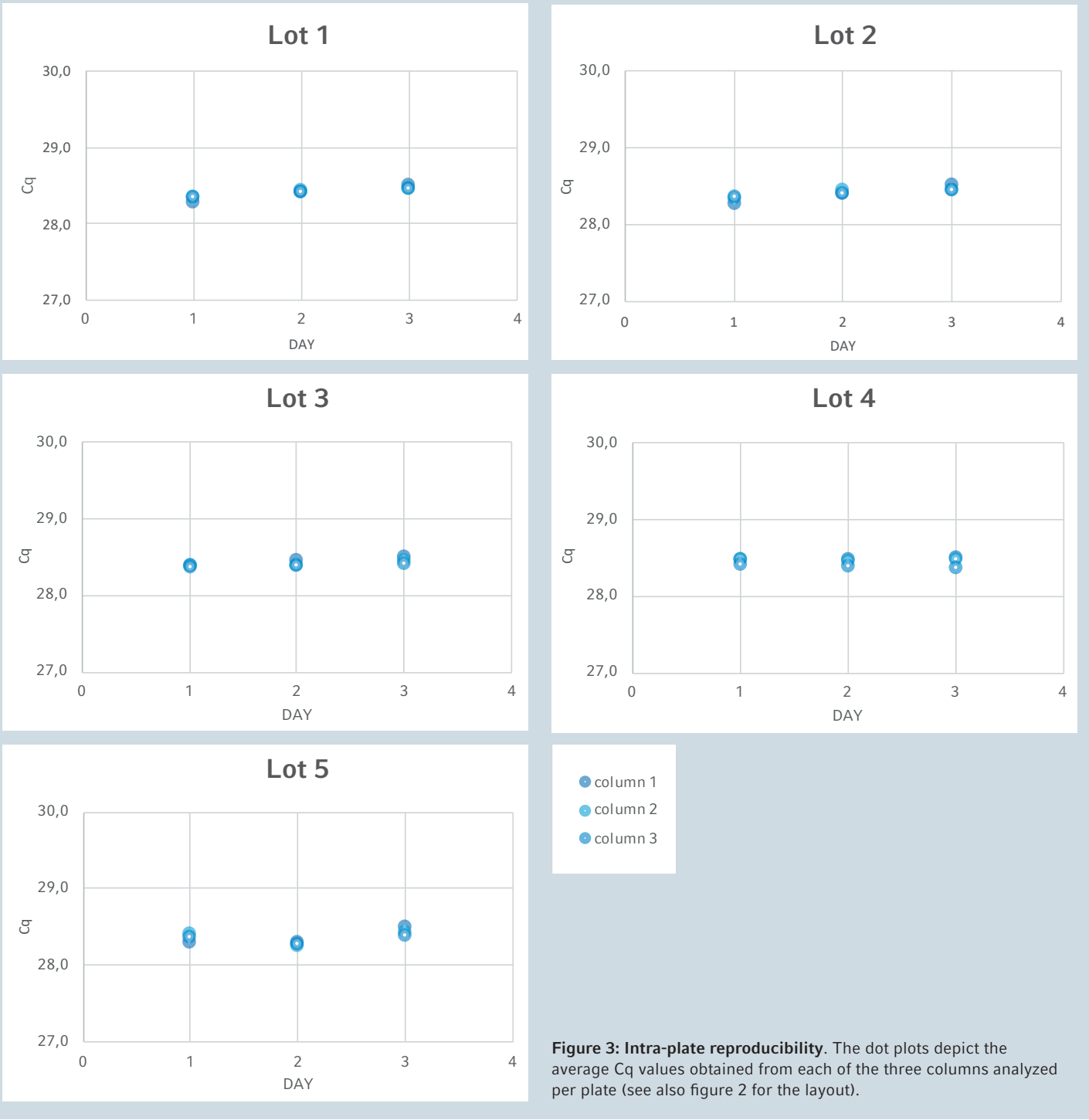


Figure 3: Intra-plate reproducibility. The dot plots depict the average Cq values obtained from each of the three columns analyzed per plate (see also figure 2 for the layout).

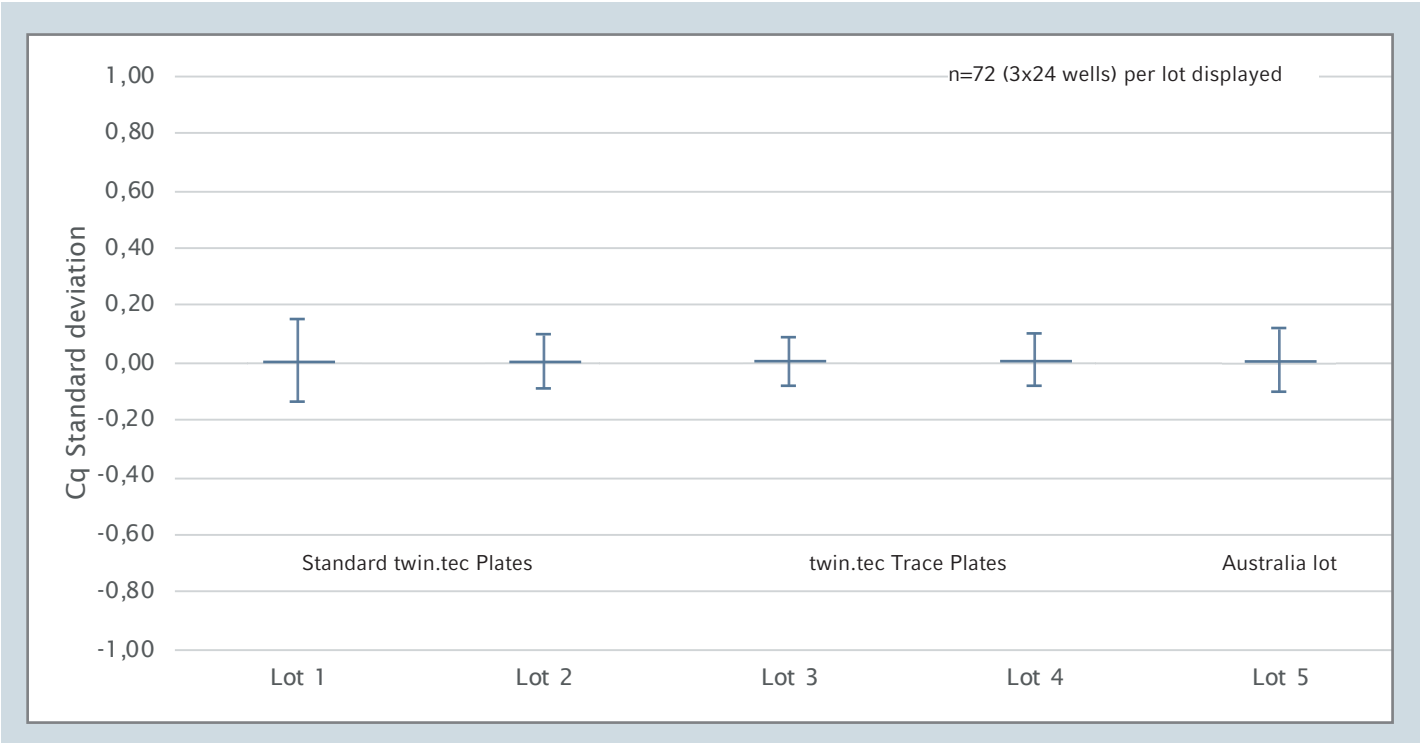


Figure 4: Inter plate reproducibility statistics. Cq standard deviation obtained for twin.tec PCR plates evaluated by lot (3 plates per lot). Per each plate 24 well were evaluated.

Conclusion

PCR plastic consumables may substantially vary in their quality and overall performance, which may negatively influence qPCR assay reproducibility. A high structural homogeneity, material purity and high-precision production process is crucial to assure the highest reproducibility of the amplification results.

In this project, we demonstrated the optimal and highly reproducible inter-lot performance of the twin.tec PCR 96 plates using a qPCR assay. The intra-plate, inter-plate homogeneity was very high with a coefficient of variation of 0.40%. The high-quality material and the state-of-the-art production process of the twin.tec PCR plates is the key factor in achieving such high inter-lot reproducibility.

This performance is independent regardless of whether different wells of the same plate (intra-plate), different plates of the same production lot (intra-lot), or plates of different lots (inter-lot) are compared. Even plates taken from a 4 years old production lot transported to Australia and back to Germany offered the same consistent performance – a demonstration of the high-quality material and high-precision, state-of-the-art production process characterizing the Eppendorf twin.tec Plates!

For scientists who transfer their established protocols to new lab facillities or expect their assays to perform reproducibly even years after initial development high quality plates can contribute to their trust in their data's reproducibility.

Ordering information

Ordering information

Description	Order no. international	Order no. North America
Eppendorf twin.tec® Trace PCR Plate 96, skirted, PCR clean, clear, 25 plates	0030 129.768	0030129768
Eppendorf twin.tec® PCR Plate 96, skirted, PCR clean, colorless, 25 plates	0030 128.648	951020401
Eppendorf twin.tec® PCR Plate 96, semi-skirted, PCR clean, colorless, 25 plates	0030 128.575	951020303

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