Internal quality assessment scheme for real-time PCR applications

H. Polin[1], M. Danzer[1], R. Haunschmid[2], K. Hofer[1], B. Fiedler[1], J. Pröll[1], C. Gabriel[1]

ÖSTERREICHISCHES ROTES KREUZ
OBERÖSTERREICH

INTRODUCTION Mandatory, laboratory accreditions ask for continuous demonstration of sufficient performance of relevant components[1]. Next to regular proficiency testing, approved quality results have to be maintained by standardized protocols and by regularly evaluation and qualification of all supplies and devices accompagnied by consistent monitoring of the assays[2]. We therefore developed a strategy of internal quality control of real-time PCR based methods for error detection.

PRINCIPLE and INTERPRETATION Two MS Excel based sheets were designed for statistical analysis of 1) supplies and 2) SPC (statistical process control). Average Crossing Points (Cps), standard deviations (SD), coefficients of variation (CV), $\bar{x}$ ± 2 SD regions (warning limits) and $\bar{x}$ ± 3 SD regions (control limits) are automatically calculated by the respective MS Excel sheet, based on five Cps entered for calibration. The calibration process is based on normally distributed data which was verified by Kolmogorov Smirnov test. Results are displayed in Shewart control charts[3]. Qualification of supplies was performed by a beta-globin PCR (Fig. 1). Cps calculated by the LightCycler Software were transferred into the MS Excel QC template for statistical analysis. The beta-globin PCR was designed for susceptibility to changes in amplification temperature of less than one degree Celsius. Localization of the mean Cp of the 150 fg/µl standard samples inside the warning control limits leads to validation of the respective real-time instrument or enzyme (Fig. 2a). Furtheron, to reach validation, the variant of coefficient (CV) based on the five replicates of the 150fg/µL standard samples must not exceed values of 5 %. For SPC, Cp data and fluorescence intensities from all types of positive and internal controls of any specific real-time assay are analyzed with MS Excel SPC template. If the generated data is acquired by localization of the control data in-between the upper and lower control limits, the assay is within statistical control (Fig. 2b). Control data outside the $\bar{x}$ ± 3 SD regions indicate that the process is out of control with the consequence to define the respective output invalid.

Fig. 2a Qualification of supplies with beta-globin PCR

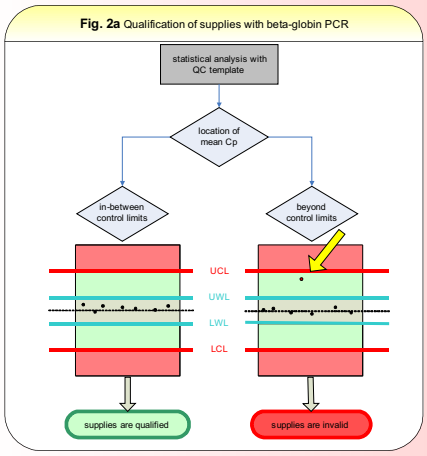


Fig. 2b SPC for diverse real-time methods

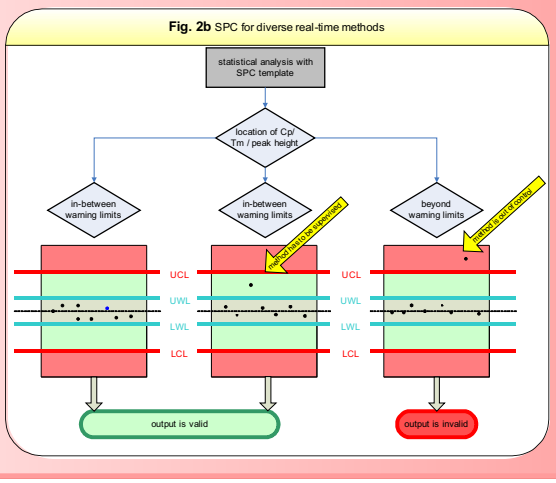
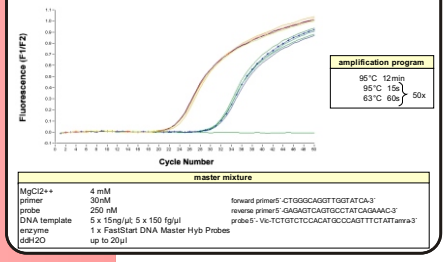


Fig. 1 beta-globin PCR



RESULTS I

The accuracy of the real-time amplification instruments was assessed by comparison to calibration data in a bi-monthly interval. By this process, one outlier out of 133 data sets was defined whose mean Cp data was located beyond the upper control limit (UCL) (Fig. 3a). Further maintenance revealed an insufficient fluorimeter device responsible for the inappropriate result. All 30 newly arrived lots of the FastStart DNA Master Hybridization Probes Kit enzyme were qualified in the same way. Our strategy is based on suggestions of the EPA[4] and CLSI[5] concerning the acquisition of quality-controlled enzymes from a commercial supplier with information about the enzyme purity provided and comparison of a new enzyme with old lots using known controls.

RESULTS I

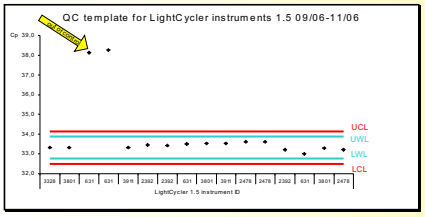


Fig. 3a During the test period, 7 different LightCycler 1.5 instruments, 9 diverse LightCycler 2.0 units and 30 different lots of the FastStart DNA Master Hybridization probes kit enzyme were evaluated.

RESULTS II

This software tool helps to visualize shifts in the diagnostic processes as shown for the internal control (IC) of bacterial 16S rDNA screening (Fig. 3b). Cps of each type of controls can be used for statistical analysis and therefore the stability and exact performance of extraction and amplification processes is guaranteed. Controls which are composed of one type of starting material have to show identical results every test. Therefore, by discrepancies of Cp data, which can be easily monitored by the graphic output of the adjusted MS Excel sheet, irregularities of PCR performance are immediately discerned.

RESULTS II

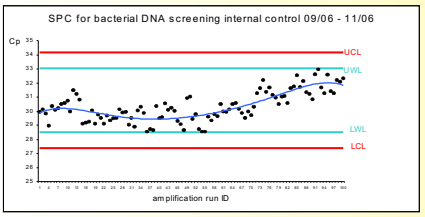


Fig. 3b SPC was applied for the daily-routine blood donor screening parameter bacterial 16S rDNA (n=150)

RESULTS III

198 data recordings of the low-positive control of HBV DNA were read into the software tool. Detection of abnormal patterns under the influence of special causes of variation were recorded in 18 (9.1%) data sets, identified in-between the upper warning and control limits demanding for enhanced monitoring of the process (Fig. 3c). 3 (1.5%) data sets outside the upper control limits indicated a loss in sensitivity due to user error or degradation of PCR reagents. Validity of the assay was verified by repetition achieving results inside upper and lower warning limits.

RESULTS III

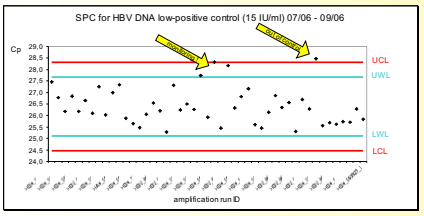


Fig. 3c SPC was applied for the daily-routine blood donor screening parameter Hepatitis B Virus (HBV) (n=198)

CONCLUSION

Leaky amplification instruments maintenance and varying performance of enzyme lots are known as common sources of error in real-time procedures. We developed a strategy for qualification of real-time assays and supplies which can be easily applied in every clinical diagnostic laboratory. Shewart control charts approved practical to visualize the variation of the process and the performance of supplies.

IMPRESSUM
[1]Red Cross Transfusion Service of Upper Austria, Linz, Austria
[2]Federal Agency for Water Management, Mondsee, Austria

Correspondence: Helene.Polin@Blutz.o.redcross.or.at

REFERENCES

1 **Ramsden S.C., S. Daly, W.-J. Geilenkeuser, G. Duncan, F. Hermitte, E. Marubini, M. Neumaier, C. Orlando, et al.** 2006. EQUAL-quant: an international external quality assessment scheme for real-time PCR. Clin Chem 52:1584-91.
2 **Burkardt H.J.** 2000. Standardization and quality control of PCR analyses. Clin Chem Lab Med 38:87-91.
3 **Shewart WA.** Economic Control of Quality of Manufactured Product. New York; D. Van Hostrand Company, Inc., 1931.
4 **United States Environmental Protection Agency (EPA).** 2004. Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples.
5 **Clinical and Laboratory Standards Institute (CLSI).** 2006. Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline. NCCLS MM03-A2.