APPLICATION NOTE No. 402

Economic DNA Determination in the Eppendorf BioSpectrometer® Fluorescence Using Qubit™ Assay kits

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Abstract

Different concentrations of dsDNA samples were determined by the Qubit dsDNA assay on the Eppendorf BioSpectrometer fluorescence. Both the UVette and the Eppendorf µCuvette were used for the measurements.
In order to determine the sample concentration, different regression possibilities of the standard curve were applied. In addition, the results were compared with measurements in the Qubit device, which were performed in parallel with the same samples. We show that the Qubit assay can be used with both the Uvette® and the Eppendorf µCuvette®. In both cases, the assays can be performed at a significantly lower volume compared to measurements in the Qubit device. Applying BioSpectrometer fluorescence and UVette requires only half of volume of the Qubit reagent, thus doubling the sample capacity of the kit.

Introduction

Besides the classic method of determining the concentrations of dsDNA via absorbance measurements at 260 nm, quantification methods based on fluorescence are well-established. It is easier to perform absorbance measurements, as these methods function without additional aids such as dyes, accuracy and detection will soon reach its limits: This is especially true where samples of very low concentrations are concerned. As such, fluorescence-based quantification is 1,000-fold more sensitive than quantification via absorbance. For example, one widely used, robust detection method is based on the quantification of dsDNA using the fluorescent dye PicoGreen® [1,2]. This method offers a broad dynamic range, and it can be employed on different fluorimeters, including the Eppendorf BioSpectrometer fluorescence.
In contrast, dyes, which are optimized for designated fluorimeters are available; these include, for example, the commonly used Qubit™ dyes which are intended for use on the corresponding Qubit fluorimeters.

The following article will describe whether, and under which conditions, Qubit reagents can be measured on the BioSpectrometer fluorescence: We describe the parameters and settings to be selected on the instrument. Two Eppendorf cuvette models, the Eppendorf UVette® and the Eppendorf µCuvette™ G1.0, were used in combination with the Qubit reagent. The results gained from these measurements were then compared to results obtained from the Qubit instrument with respect to accuracy and precision. For these measurements, the "Qubit dsDNA High Sensitivity Kit" for the determination of dsDNA samples in the range between 0 and 500 ng/mL was selected. In addition to comparison with the Qubit instrument, method optimizations were performed on the BioSpectrometer which included a variety of standard curve regression options. These were subsequently compared to one another with respect to the results obtained.

Materials and Methods

Materials

> BioSpectrometer fluorescence
> Qubit™ 2.0
> UVette
> Eppendorf µCuvette™
> Qubit dsDNA HS Assay Kit
> MixMate®
> Qubit Assay Tubes
> Eppendorf Safe-Lock Tubes, 0.5 mL

Measurements of standards

Qubit:
For the Qubit instrument, the dsDNA standards supplied in the kit were prepared and used in accordance with the manufacturer's instructions (0 and 500 ng/mL). Operation of the Qubit was carried out in accordance with the experimental instructions included in the kit.

BioSpectrometer:
The standards provided in the Qubit kit were also used with the BioSpectrometer fluorescence. Additional concentrations were prepared through dilutions using the Qubit buffer (0, 100, 250, 500 ng/mL), thus allowing the use of different regression options on the BioSpectrometer.
In addition to the 2-point regression (linear interpolation), linear and quadratic regressions were employed for sample quantification, and they were compared to the results obtained with the Qubit instrument.

Samples:
Four sample concentrations were generated using the dsDNA standard included in the kit. The stock concentration of the standard was 10 µg/mL, which was diluted to the following concentrations of dsDNA:

Initial concentration [µg/mL]	**Final concentration [ng/mL]** (following 1:20 dilution in Qubit reagent)
10	500
5	250
2	100
1	50

Five individual dilutions were prepared for all sample concentrations and measured (5 replicates).

Preparation of the samples and standards

Qubit:
10 µL of samples and standards, respectively, were added to 190 µL of measurement buffer (Σ=200 µL).

BioSpectrometer:
When using the UVette, 5 µL of sample and 5 µL of standard, respectively, were diluted in 95 µL of measurement buffer (Σ=100 µL). The dilutions were prepared directly inside the UVette and mixed by pipetting up and down. Samples and standards could then be measured directly in the UVettes.

In contrast, measurements which were carried out in the µCuvette required the transfer of only 2 µL of sample or standard and 38 µL of Qubit buffer (Σ=40 µL) into 0.5 mL Safe-Lock tubes, followed by thorough mixing (MixMate). A volume of only 5 µL is sufficient for the µCuvette.
The total sample volume of 40 µL for measurement in the µCuvette was selected to ensure error-free pipetting of samples and standards, which cannot be guaranteed for volumes below 2 µL. The required 20-fold dilution thus results in a total volume of 40 µL, which deviates considerably from the actual volume required for the measurement (5 µL).
Prior to measurement, all samples and standards should always be checked for air bubbles, and it is important to ensure that the cuvette shaft remains closed during the measurement process.

Operation

Qubit:
Operation of the Qubit was in accordance with the instructions provided in the kit manual.

BioSpectrometer:
The pre-programmed Qubit HS method was used for measurements in the BioSpectrometer (figure 1).

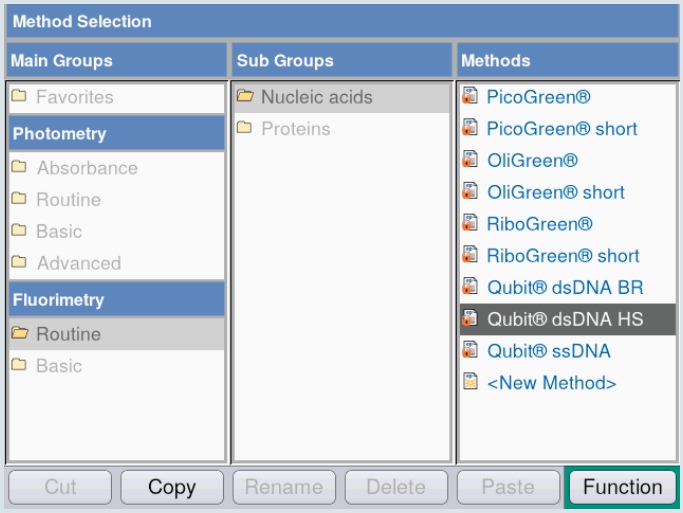

Figure 1: Selection of the pre-programmed Qubit method

The number of standards and their respective concentrations must be defined in the section "Check Parameters" prior to sample quantification (figures 2A and 2B).

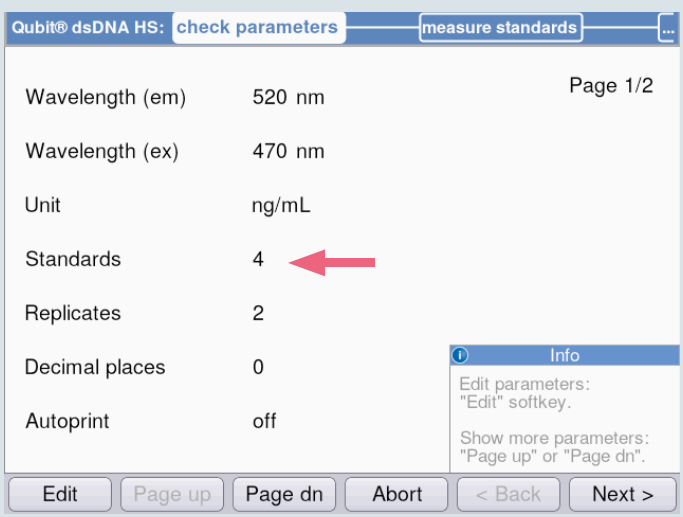

Figure 2A: Definition of the number of standards (red arrrow)

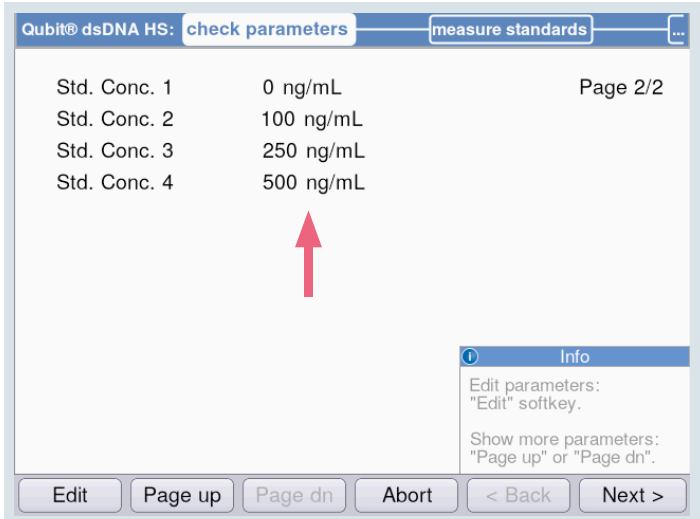

Figure 2B: Definition of the standard concentration.

The regression analysis applied to the standard curve may be adjusted via the function "Curve Fit" (figure 3).

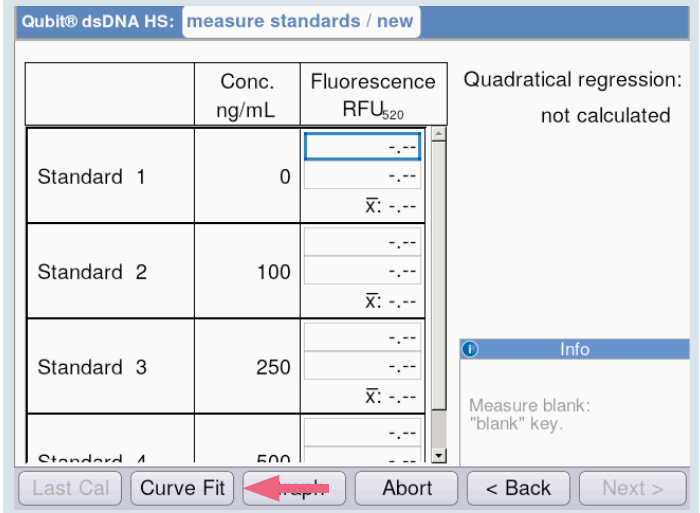

Figure 3: Regression analyses may be adjusted via the function "Curve Fit" (red arrow).

Results and Discussion

A) Qubit HS kit used in combination with the UVette

Different regression analyses were applied to the measurements performed in the UVette as well as the µCuvette. Figure 4 (A - C) shows the respective standard curves resulting from measurements using the UVette.

The samples (500, 250, 100, 50 ng/mL) were quantified using each of the regression analysis methods introduced above. The final sample volume was 100 µL in all cases.

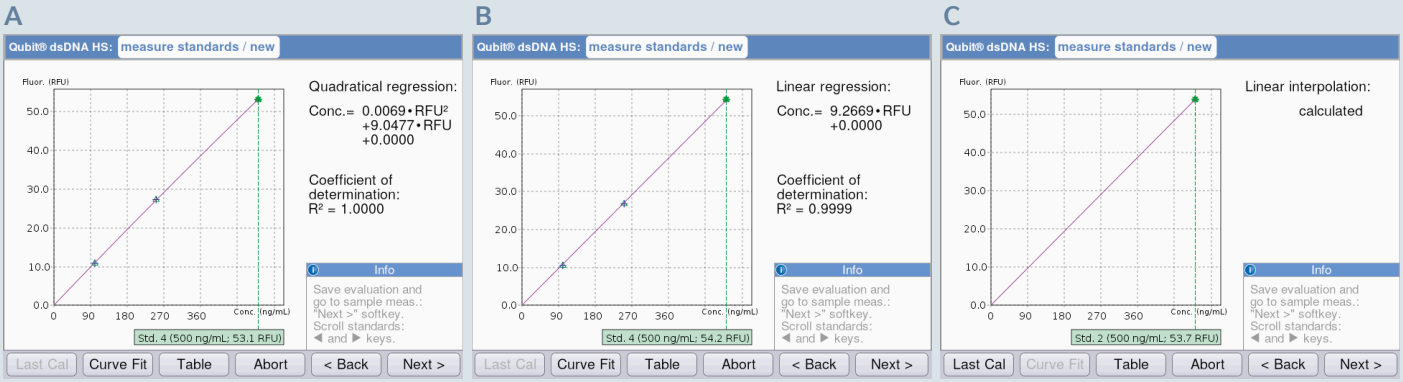

Figure 4: Analyses of the standard curve obtained from measurements using the UVette. Evaluated via A: Quadratic regression, B) Linear regression and C) Linear interpolation.

Figure 5 presents an overview of the precision values achieved for all regression analyses, in comparison with the results obtained in the Qubit.

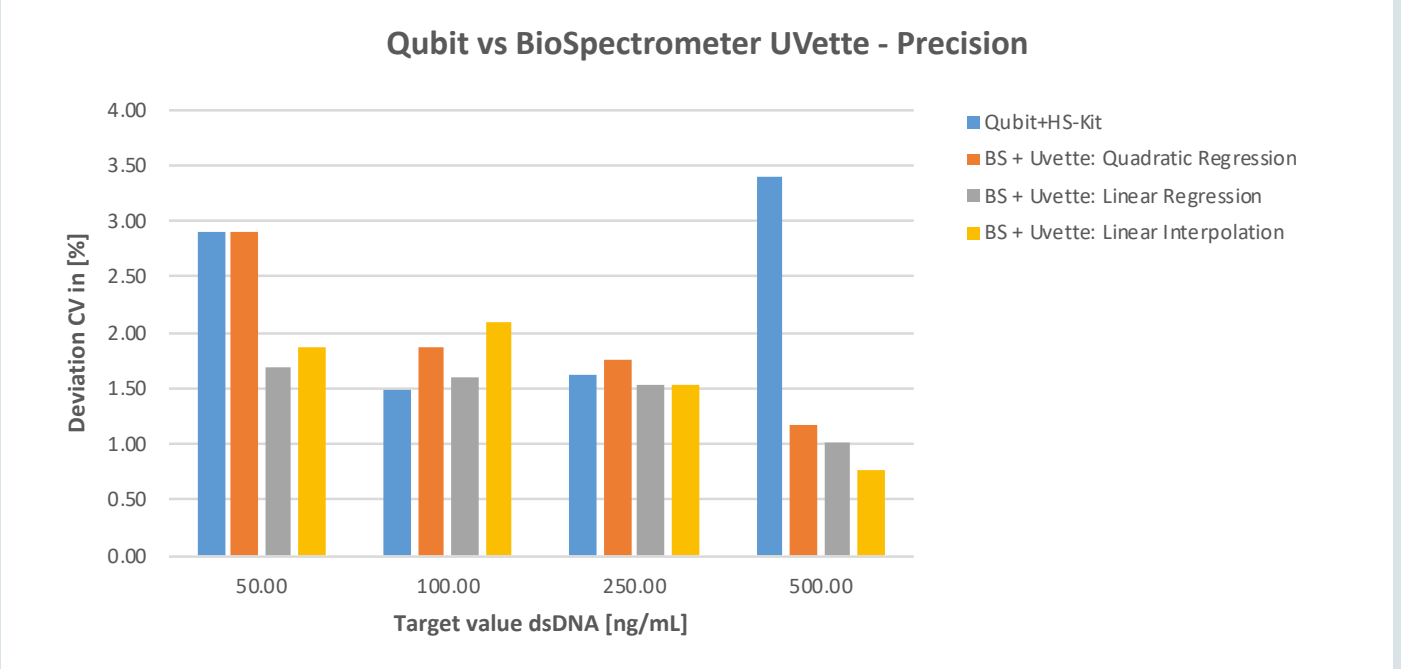

Figure 5: Precision of the respective series of measurements with respect to the expected sample concentrations as measured in the UVette

In addition to precision, the deviation from the target value was also determined. Figure 6 shows the deviation results after applying the different methods of regression analysis to the measurements obtained with the BioSpectrometer as well as the Qubit.

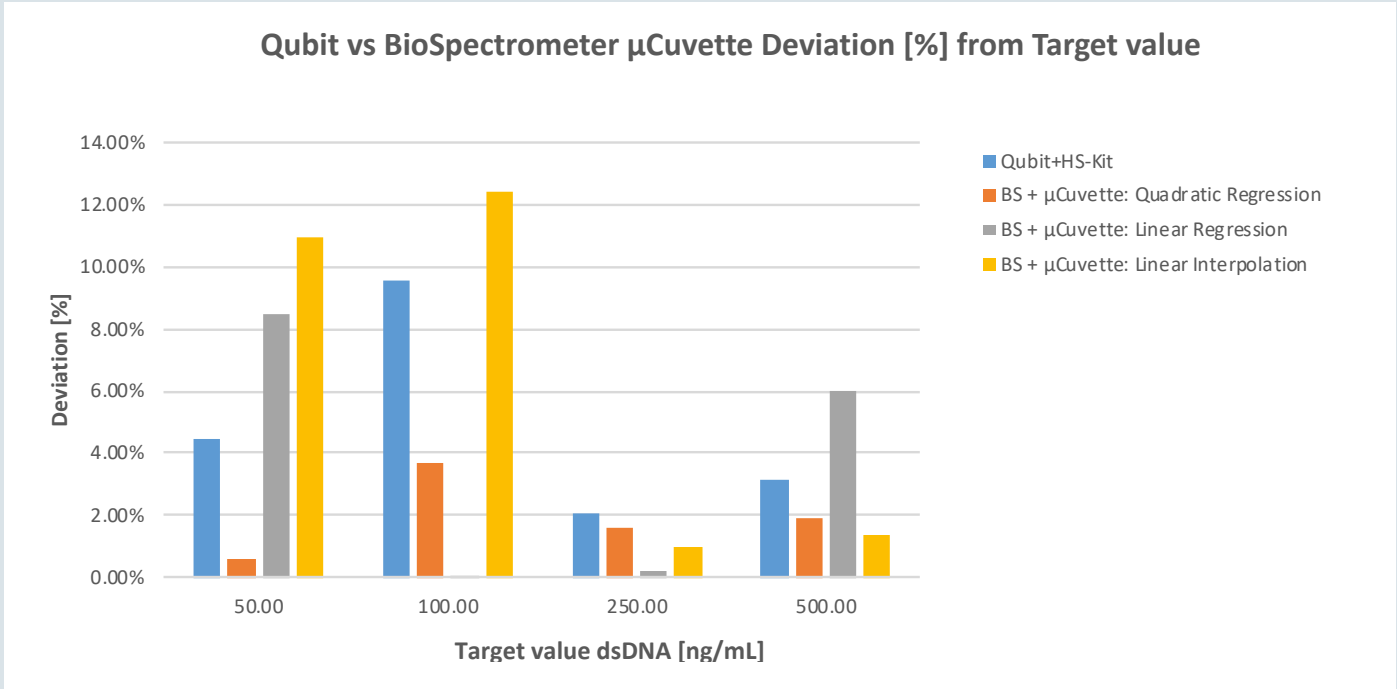

Figure 6: Deviations of the measurement series with respect to the expected sample concentrations as measured in the UVette.

B) Qubit HS kit used in combination with the µCuvette

Further to the UVette, the suitability of the Eppendorf µCuvette in combination with the Qubit HS kit was investigated. Figure 6 (A,B,C) shows the respective standard curves for quantifications carried out using the µCuvette.

The samples (500, 250, 100, 50 ng/mL) were quantified using each of the regression analysis methods introduced above. The final sample volume was 40 µL in all cases.

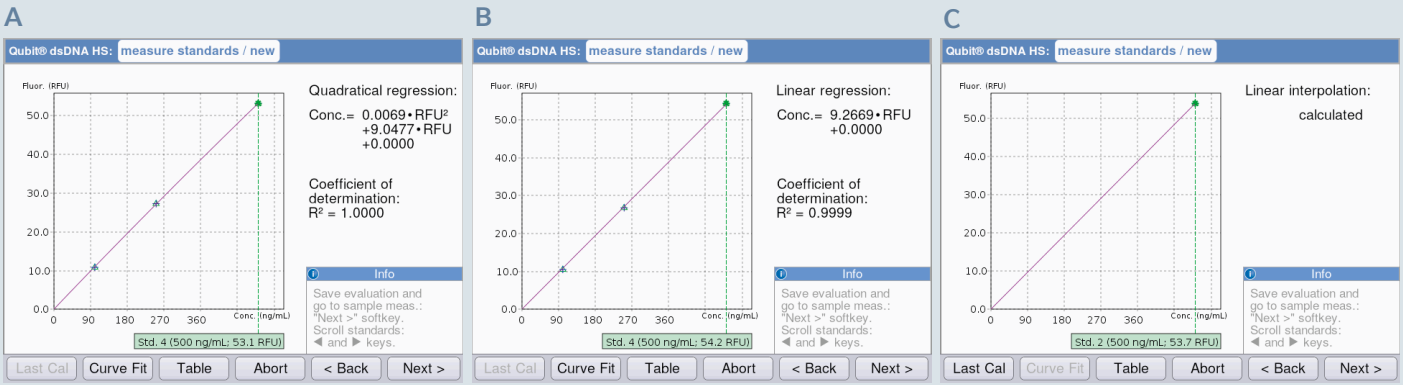

Figure 7: Analyses of the standard curves generated with the µCuvette.
Evaluated via A): Quadratic Regression, B) Linear Regression and C) Linear Interpolation.

Figure 8 shows an overview of the precision values achieved for all regression analyses in relation to the results obtained with the Qubit.

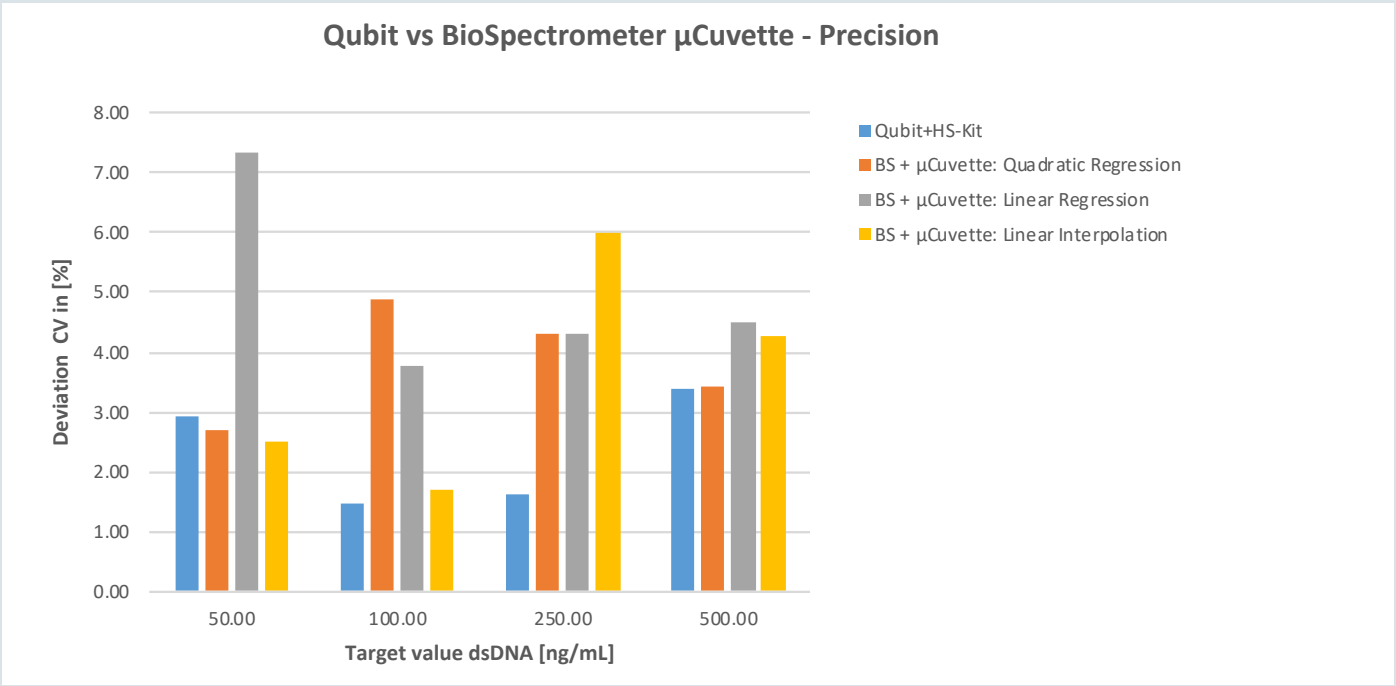

Figure 8: Precision of the respective series of measurements with respect to the expected sample concentrations as measured in the µCuvette.

In addition to precision, the deviation from the target value was also determined. Figure 9 shows the deviation results after applying the different methods of regression analysis to the measurements obtained with the BioSpectrometer as well as the Qubit device.

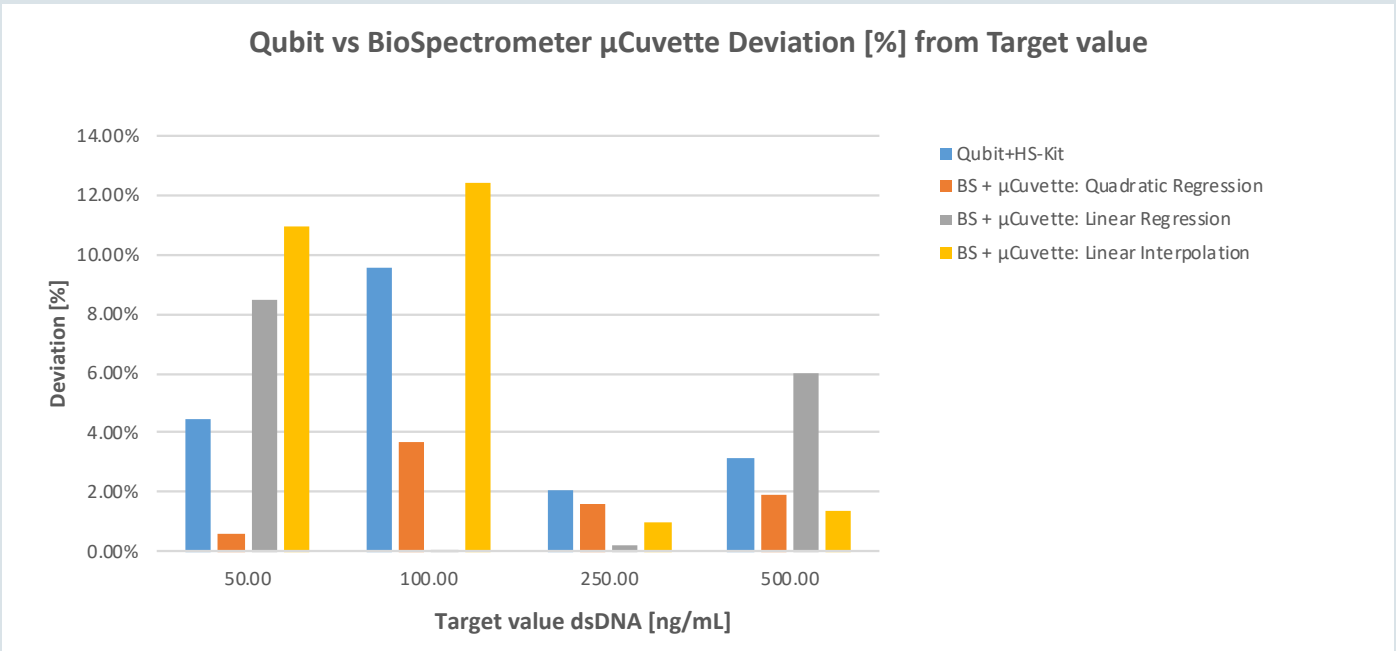

Figure 9: Deviations of the measurement series with respect to the expected sample concentrations as measured in the µCuvette.

Conclusion

Use of the UVette in combination with the HS kit

It was shown that the BioSpectrometer fluorescence, in combination with the UVette, was used successfully for fluorescence-based quantification of dsDNA, as the results were similar to those obtained with the Qubit instrument. The standard curve showed a linear course; therefore it was inconsequential which regression method was selected (figures 5 + 6). Thus, in order to save time and reagent, linear interpolation (2-point method) is generally sufficient for standard evaluations (3). The significant advantage of the combined use of BioSpectrometer fluorescence and UVette: Only half of the Qubit reagent volume is required, thus doubling the sample capacity of the kit.

Use of the µCuvette in combination with the HS kit

The µCuvette, too, can be used in the BioSpectrometer fluorescence in combination with the Qubit HS kit (3). In line with the standard curves obtained with the UVette, a discernible linear trend was also achieved with the µCuvette, and the reduction of the total volume to 40 µL enabled saving even more reagent compared to the UVette. The variations of the measurements were slightly elevated compared to those observed with both the UVette and the Qubit device, which may be explained by taking into consideration the higher systematic error of the pipette which is observed when pipetting smaller volumes. The best results were achieved with the use of four standards and applying quadratic regression analysis.

Recommendation

The Qubit HS kit can be used in combination with both cuvettes, the UVette most likely being considered more convenient. This is due to the fact, that larger volumes are more easily pipetted, which, in turn, renders the measurement less susceptible to error and the results more accurate.

A significantly larger sample volume needed to be prepared for the µCuvette (40 µL) than was actually required for the measurements (5 µL). Further reduction of the sample volume was not useful as it would further increase the error rate of the measurement system.

Use of additional Qubit kits

In addition to the HS kit, Qubit offers the BR Assay Kit for the determination of dsDNA; this widely applicable kit is capable of quantifying 10-fold higher concentrations of dsDNA samples than the HS kit (0-5000 ng/mL BR vs. 0-500 ng/mL HS). With respect to the volumes, the measurement procedure for the BR kit is identical to that of the HS kit. In contrast to the HS kit, however, we recommend the use of four standards and quadratic regression analysis when employed in the BioSpectrometer, as these standards did not follow a linear curve as their concentrations increased, in relation to fluorescence measured (figure 10). With this kit, superior results were obtained using the UVette as compared to the µCuvette. A detailed description of the measurement procedure is available in the respective Short Protocol (4).

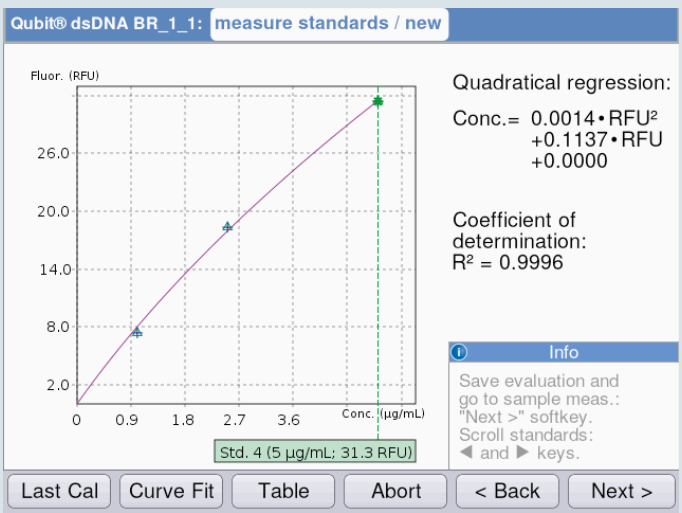

Figure 10: Standard curve of the Qubit BR kit as measured in the BioSpectrometer.

Literature

[1] Armbrecht, M. Fluorimetric Determination of dsDNA Concentrations via 2-point Calibration. Eppendorf Short Protocol No. 18
[2] Armbrecht, M, Gloe, J, Goemann, W. Determination of nucleic acid concentrations using fluorescent dyes in the Eppendorf BioSpectrometer® fluorescence. Eppendorf Application Note No. 271 (2013)
[3] Armbrecht, M. Using the Qubit™ dsDNA HS Kit on the Eppendorf BioSpectrometer® fluorescence. Eppendorf Short Protocol No. 36
[4] Armbrecht, M. Using the Qubit™ dsDNA BR Kit on the Eppendorf BioSpectrometer® fluorescence. Eppendorf Short Protocol No. 37.

Ordering information

Description	Order no. international	Order no. North America
Eppendorf BioSpectrometer® fluorescence 230 V/50-60 Hz, electrical plug Europe, additional electrical connection variants available 120 V/50-60 Hz, electrical plug North America	6137 000.006 6137 000.014	 6137000014
Eppendorf µCuvette® G1.0, Microvolume measuring cell for Eppendorf BioPhotometer and BioSpectrometer	6138 000.018	4987000118
UVette® routine pack 220 nm – 1 600 nm Eppendorf Quality purity, re-sealable box, 200 pcs	0030 106.318	6138000018
MixMate®, incl. 3 tube holders: PCR 96, 0.5 mL, 1.5/2.0 mL, 100 – 120 V/50 – 60 Hz (US)	5353 000.014	022674200

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