

Applications

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Reproducible photometric determination of DNA concentrations using the Eppendorf UVette® in the Eppendorf BioPhotometer plus™

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Abstract

DNA concentrations are typically determined photometrically with the help of re-usable quartz cuvettes. This article will demonstrate that these measurements yield equally good results when performed using single-use plastic cuvettes like the Eppendorf UVette. To this end, measurements of different DNA concentrations were carried out in the UVette, using the Eppendorf BioPhotometer plus. The reproducibility of this system was demonstrated with the help of the calculated coefficient of variation. Series of measurements were generated using the 2 mm light path, as well as the 10 mm light path, of the Eppendorf UVette, and compared to a quartz cuvette.

Introduction

It is common practice to perform photometric nucleic acid determinations in cuvettes made from quartz, as it is commonly assumed that plastics are not UV-transparent and therefore, not suitable for nucleic acid measurements. As we will show in this article, photometric measurements in the UV range can be performed reproducibly in the Eppendorf UVette, as the special plastic is UV-transparent. Thus, the Eppendorf UVette is one of the few single-use cuvettes made from plastic which may be used for measurements in the UV-range.

Single use cuvettes such as the Eppendorf UVettes have an advantage over re-usable cuvettes made from quartz in that the sample to be measured cannot be contaminated by preceding samples. In addition, a sample volume of 50 µL is sufficient for reproducible measurements in the UVette. The standard light path for the UVette is 10 mm. However, highly concentrated solutions may be measured in the additional light path of 2 mm (see figure 1). For this application, the UVette is simply rotated by 90°. Thus, the UVette offers the flexibility to measure with two different light paths, where normally two different cuvettes would be used. This feature allows for a considerable expansion of the measurable range of sample concentrations.

This article shows that reproducible measurements in the UV range may be carried out using both light paths.

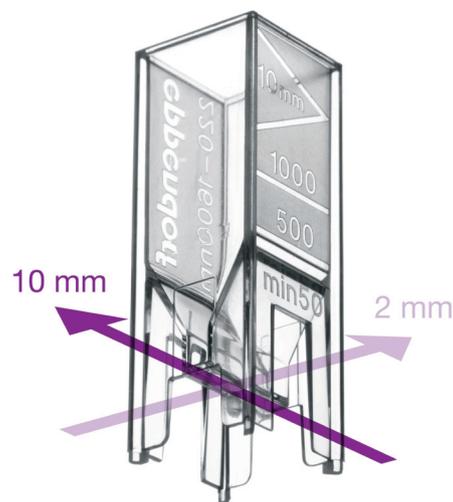


Figure 1: Eppendorf UVette

Two light paths are available for photometric measurements, combined in one cuvette: the standard 10 mm light path (purple) and a 2 mm light path (violet).

Materials and Methods

1) Verification of reproducibility via measurements in the UV range using the Eppendorf UVette, employing both possible light paths of 2 mm and 10 mm.

Materials

Eppendorf BioPhotometer plus
 UVettes
 BioPhotometer Data Transfer Software
 Human genomic DNA (Promega, approx. 200 ng/μL (manufacturer's specifications), measured approx. 238 ng/μL)
 Water (molecular biology grade), pH 7.0
 Eppendorf Research Pipettes
 epT.I.P.S.
 Thermo printer DPU 414
 Hellma Ultra micro cuvette made from quartz glass (path length 10 mm, QS105.202)

Method

2 serial dilutions each were generated using the human genomic DNA, where the stock solution in each serial dilution was diluted 1:2, 1:4 and 1:8. Each dilution step of each serial dilution was measured 10 times. The undiluted solution and the 1:2 dilution were measured using the 2 mm light path, whereas the 1:4 and 1:8 diluted DNA solutions were measured in the 10 mm light path.

For the purpose of comparing reproducibility, the latter dilutions were also measured in cuvettes made from quartz glass.

The use of the respective path length was taken into consideration during programming of the parameters in the BioPhotometer plus (fig. 2). The 10 measurements per dilution series were performed sequentially. A mean value was determined for each series of measurements, as well as the maximum deviation from the mean and the coefficient of variation.

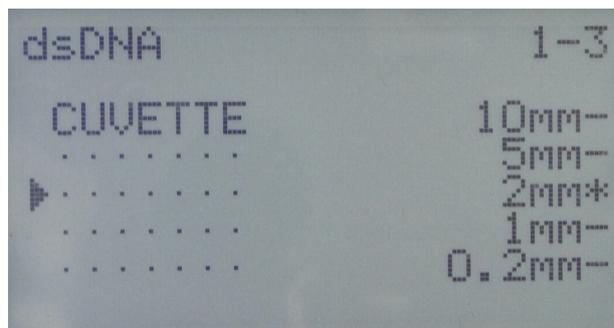


Fig. 2: Programming of the 2 mm light path in parameters for the method dsDNA

Results and Discussion

1) Reproducibility of photometric DNA determinations using Eppendorf UVettes and the BioPhotometer plus

In order to verify the reproducibility of photometric DNA measurements in Eppendorf UVettes, measurements were carried out in two concentration ranges. To this end, DNA samples were measured ten times in different cuvettes. For samples with concentrations above 100 ng/μL the 2 mm light path was used. Samples of lower concentration were measured using the 10 mm light path of the UVette, as well as a quartz glass cuvette for comparison.

The absorption results at 230, 260 and 280 nm are displayed for each series of measurements, along with the DNA concentration. The 340 nm results are not displayed, as this experiment was performed with ultra pure DNA, thus rendering impurities highly improbable.

For the purpose of determining reproducibility, the mean value was calculated, as well as the standard deviation of each measured series, and the coefficient of variation. The series of measurements were determined using the method dsDNA on the BioPhotometer plus, as this instrument allows for clear simultaneous display of all parameters relevant for DNA measurements. The results are shown in tables 1A through 1D.

Table 1D: Result of measurements using 1:8 diluted DNA solutions (10 mm light path) - UVette

Wavelength [nm]	1. UVette 10 mm path length			Concentration [ng/μL]	2. UVette 10 mm path length			Concentration [ng/μL]
	230	260	280		230	260	280	
Sample 1	0.344	0.594	0.322	29.7	0.345	0.593	0.321	29.7
Sample 2	0.345	0.595	0.322	29.7	0.344	0.594	0.32	29.7
Sample 3	0.345	0.596	0.322	29.8	0.344	0.594	0.321	29.8
Sample 4	0.345	0.596	0.322	29.8	0.344	0.595	0.321	29.8
Sample 5	0.345	0.594	0.322	29.7	0.344	0.595	0.322	29.8
Sample 6	0.344	0.595	0.322	29.8	0.343	0.595	0.322	29.8
Sample 7	0.346	0.597	0.324	29.8	0.344	0.595	0.322	29.8
Sample 8	0.346	0.597	0.323	29.8	0.345	0.597	0.322	29.8
Sample 9	0.346	0.598	0.325	29.9	0.344	0.597	0.322	29.8
Sample 10	0.346	0.597	0.323	29.9	0.344	0.596	0.322	29.8
Mean	0.345	0.596	0.323	29.790	0.344	0.595	0.322	29.780
Standard deviation	0.0006	0.0011	0.0008	0.0540	0.0004	0.0009	0.0006	0.0320
Coefficient of variation (CV)	0.2 %	0.2 %	0.3 %	0.2 %	0.2 %	0.2 %	0.2 %	0.1 %

Table 1E: Result of measurements using 1:4 and 1:8 diluted DNA solutions (10 mm light path) – quartz cuvette

Wavelength [nm]	Quartz cuvette 10 1. mm path length (1:4 diluted DNA)			Concentration [ng/μL]	Quartz cuvette 10 2. mm path length (1:8 diluted DNA)			Concentration [ng/μL]
	230	260	280		230	260	280	
Sample 1	0.677	1.156	0.642	57.8	0.341	0.584	0.327	29.2
Sample 2	0.678	1.156	0.642	57.8	0.341	0.583	0.326	29.15
Sample 3	0.677	1.156	0.643	57.8	0.341	0.584	0.327	29.2
Sample 4	0.675	1.155	0.641	57.75	0.341	0.585	0.328	29.25
Sample 5	0.677	1.155	0.641	57.75	0.341	0.584	0.327	29.2
Sample 6	0.677	1.157	0.642	57.85	0.342	0.585	0.327	29.25
Sample 7	0.678	1.156	0.642	57.8	0.343	0.585	0.328	29.25
Sample 8	0.677	1.157	0.643	57.85	0.341	0.584	0.328	29.2
Sample 9	0.678	1.156	0.643	57.8	0.343	0.586	0.33	29.3
Sample 10	0.678	1.156	0.643	57.8	0.344	0.586	0.329	29.3
Mean	0.677	1.156	0.632	57.800	0.342	0.585	0.328	29.23
Standard deviation	0.0006	0.0004	0.0006	0.0200	0.0010	0.0008	0.0009	0.0400
Coefficient of variation (CV)	0.1 %	0.1 %	0.1 %	0.1 %	0.3 %	0.2 %	0.4 %	0.2 %

The series of measurements using high DNA concentrations (undiluted and diluted 1:2), performed in the 2 mm light path, show a coefficient of variation (CV) of 0.3 % to 0.5 % for all wavelengths. The standard deviation of the mean is between 0.001 A and 0.002 A.

For the 1:4 and 1:8 diluted DNA solutions, the CV of the respective series of measurements falls between 0.1 % and 0.3 %, and the standard deviation is 0.001 A.

Table 1E shows that the standard deviations obtained with quartz cuvettes are similarly low.

The results clearly show that reproducible measurements in the UV range are equally possible in Eppendorf UVettes made from plastic as they are in quartz cuvettes. This is true for the 2 mm as well as the 10 mm light path.

With regards to reproducibility, the photometric accuracy of measured results obtained in the BioPhotometer plus may be verified using a set of calibration filters, the Secondary UV-VIS-Filter Set [1]. For this purpose, 3 broad range filters are used which yield an absorption of approximately 0.2 A at 260 nm (filter A1), 0.8 A (filter A2) and 1.4 A (filter A3). During calibration, filter A1 may show a maximum CV of 3 %, filter A2 may show a maximum CV of 1 % and filter A3 may show a maximum CV of 1.5 %. Figure 3 summarizes the CV values obtained from the series of measurements using the UVette and the glass cuvettes. Figure 3 shows that the coefficients of variation measured in the UVette as well as in the quartz cuvettes are clearly below the maximum permitted CVs of the UV-VIS filter set.

These results underline the reproducibility of measurements obtained using the UVette.

Conclusion

This article was able to demonstrate that single-use UV cuvettes made from plastic, such as the Eppendorf UVette, are capable of producing reproducible measurements in the UV range. As verified by the series of measurements using dsDNA, the deviations for both the 2 mm light path, as well as the 10 mm light path, are well within the technical specifications of the BioPhotometer plus, which was used for these measurements. The fact that both path lengths showed high reproducibility during these measurements underlines the flexible application possibilities of the UVettes during measurements in the UV range. In addition, the possibility to measure higher concentrations using the same cuvette and a shorter light path is to date a unique feature among plastic cuvettes.

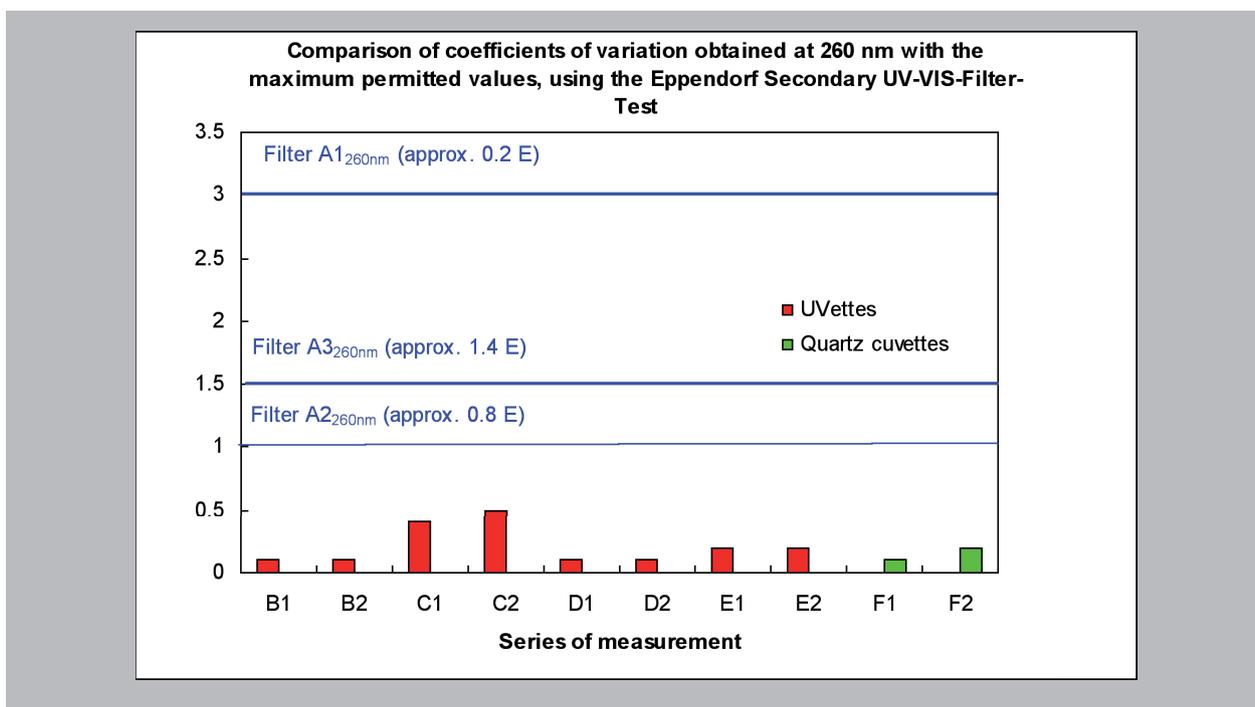


Fig. 3: Measured CVs obtained from series of measurements UVette and quartz cuvettes in comparison to the maximum permitted CVs of the calibration filters (Secondary UV-VIS Filter Set).

Blue lines: permitted coefficients of variation of the calibration filters A1, A2, A3 at 260 nm

B1-E2: CVs of the series of measurements obtained from the measurements using the UVette at 260 nm

F1-F2: CVs of the measurements obtained with quartz cuvettes at 260 nm.

Literature

- [1] Eppendorf UserGuide No. 10, Evaluating the functionality of the Eppendorf BioPhotometer plus using the Secondary UV-VIS Filter Set. www.eppendorf.com

Ordering Information

Description	Order No. International	Order No. North America
Eppendorf BioPhotometer plus™, 230 V / 50 - 60 Hz	6132 000.008	-
Eppendorf BioPhotometer plus™, 120 V / 50 - 60 Hz	-	952000006
Thermo printer DPU 414 Incl. adapter and printer cable 230 V	6131 011.006	-
Thermal Printer DPU 414, serial printer incl. power supply 120 V/60 Hz unit and printer cable	-	952010140
Printer Paper, 5 rolls	0013 021.566	952010409
UVette® Original Eppendorf plastic cuvettes, individually wrapped, Usable directly in the BioPhotometer, certified RNase-, DNA and protein free, pack of 80	0030 106.300	952010051
UVette® routine pack Eppendorf Quality purity, re-sealable box Pack of 200	0030 106.318	952010069
Cuvette rack For 16 cuvettes	4308 078.006	940001102
BioPhotometer Data Transfer Software	6132 854.007	952000500
Secondary UV-VIS-Filter Test filter set for verification of photometric and wavelength accuracy (in accordance with NIST)	6131 928.007	952010221

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