Comparison of Quant-iT and Qubit DNA quantitation assays for accuracy and precision

Accurate DNA quantitation is essential for many molecular biology applications, including sequencing and qPCR, and researchers have a variety of choices in DNA quantitation kits and devices. This document compares two of our methods for the quantitation of DNA: the Invitrogen™ Quant-iT™ PicoGreen™ dsDNA assay and the Invitrogen™ Qubit™ dsDNA assays. The Quant-iT PicoGreen dsDNA assay is well-adapted to high-throughput use, typically in a 96-well plate. Samples processed using the Quant-iT PicoGreen dsDNA Assay Kit are read with a standard fluorescence microplate reader, and the data are plotted with a graphics software package of the user's choice. Qubit dsDNA assay kits are convenient for fewer samples, since the samples are read one at a time. The Qubit assays are available as a HS (high sensitivity) assay kit and a BR (broad range) assay kit. For both Qubit assays, samples are processed and data are plotted using the Invitrogen™ Qubit™ Fluorometer, which has built-in software for DNA quantitation. The algorithms employed by the Qubit Fluorometer are optimized for the DNA concentration ranges and detection reagents used in each assay type.

Table 1. Comparison of the Quant-iT PicoGreen dsDNA assay to the Qubit dsDNA HS and BR assays.

	Quant-iT PicoGreen dsDNA assay with linear curve fitting	Qubit dsDNA HS and BR assays with nonlinear curve fitting
Number of samples	20–20,000	<20
Data analysis	Performed by user	Performed by Qubit Fluorometer
Precision	For 0.5–1,000 ng/mL: CV <5%	HS: 0.5–500 ng/mL: CV <5% BR: 0.01–5 µg/mL: CV <5%
Accuracy to expected results	For 0.5–10 ng/mL: accuracy varies* For 50–1,000 ng/mL: accuracy within 15%	HS: For 1–500 ng/mL: accuracy within 15% BR: For 0.01 µg/mL: accuracy within 27%; for 0.02–5 µg/mL: accuracy within 15%

* For the PicoGreen assay with sample concentrations <10 ng/mL in the assay tube, use low-concentration standards or use a nonlinear standard curve as shown in Figure 1.

Summary

The Quant-iT PicoGreen dsDNA assay was compared to the Qubit dsDNA HS and Qubit dsDNA BR assays for accuracy and precision (Table 1). In this comparison, the Quant-iT PicoGreen dsDNA assay data were evaluated against a linear standard curve, whereas the Qubit dsDNA HS and BR assay data were analyzed on the Qubit Fluorometer using an algorithm based upon nonlinear curve fitting (Figure 1).

- All three assays showed good precision (CV <5%) and good accuracy above 10 ng/mL of DNA (in the Qubit assay tube or microplate well).
- At the low end of the concentration range (<10 ng/mL), the Qubit dsDNA HS and BR assays gave better accuracy and precision than the Quant-iT PicoGreen dsDNA assay due to the use of highly optimized algorithms.
- The accuracy of the Quant-iT PicoGreen dsDNA assay can be compromised at low concentrations when data across the entire dynamic range are used to generate a straight line (Figure 1).
- To achieve equivalent accuracy with the Quant-iT PicoGreen assay for dsDNA concentrations below 10 ng/mL, one can either optimize the standard curve analysis to better fit the data or, if a broad detection range is not required, one can use low concentration range DNA standards. The current Quant-iT PicoGreen dsDNA assay kit protocol provides details for using low concentration range DNA standards.

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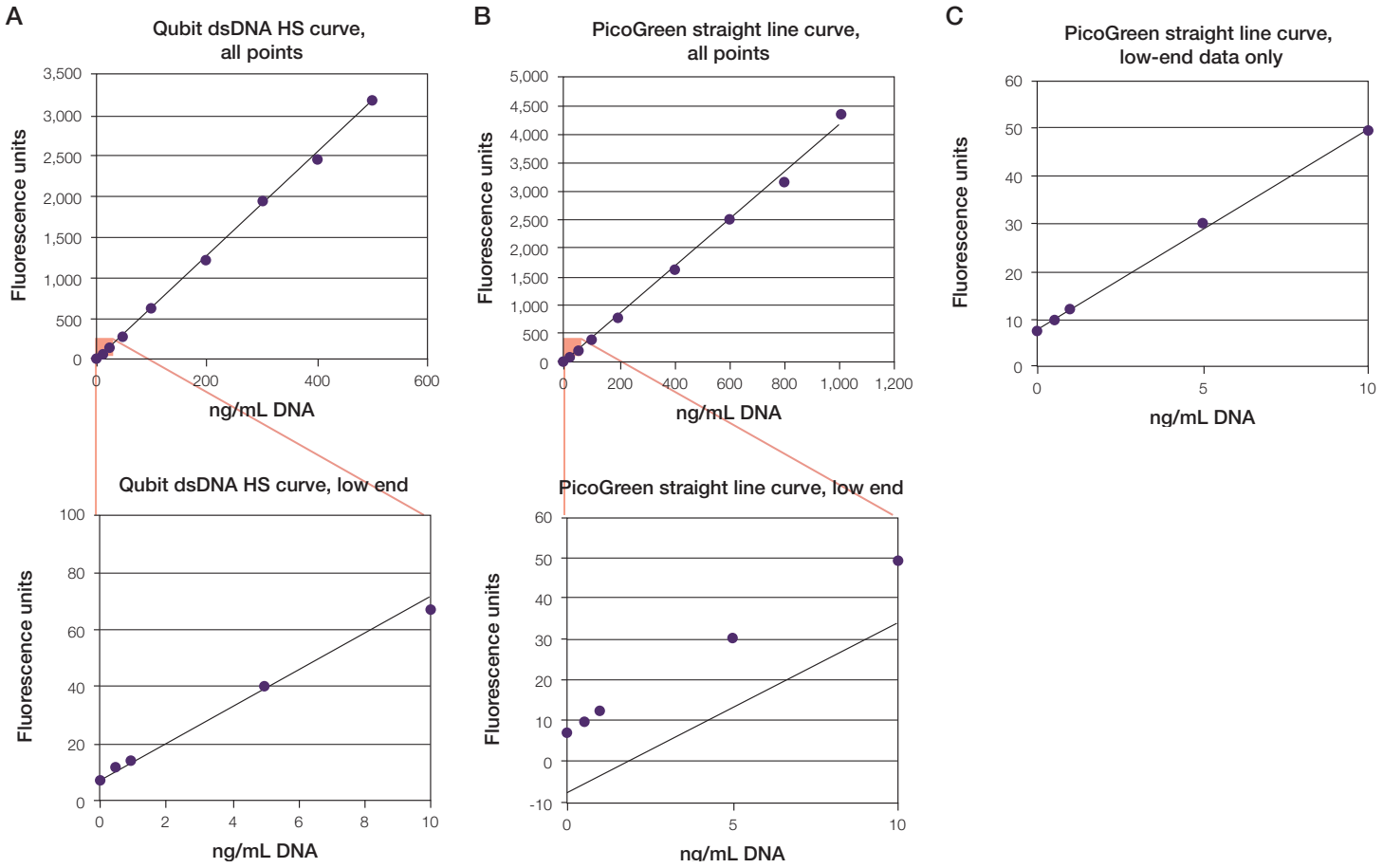


Figure 1. A dsDNA HS nonlinear standard curve used by the Qubit Fluorometer (A), compared to linear curves fitted to all data points (B) or only the low-end data points (C) of a Quant-iT PicoGreen dsDNA assay standard series.

Methods and results

Quant-iT PicoGreen dsDNA assay

The Quant-iT PicoGreen dsDNA assay was performed according to the kit instructions. Samples ranging in concentration from 0 ng/mL to 1,000 ng/mL were measured in quadruplicate, and the results are shown from the same data plotted in two different ways (Tables 2 and 3). Table 2 summarizes results of the Quant-iT PicoGreen dsDNA assay when plotted on a straight line. Table 3 summarizes the same results plotted on the Qubit dsDNA HS assay's nonlinear algorithm line. Figure 2 shows analyses of the quantitation data derived using two different standard curves.

Qubit dsDNA HS assay

The Qubit dsDNA HS assay was performed according to the kit instructions. Samples ranging in concentration from 0 ng/mL to 500 ng/mL were measured in triplicate. Figure 3 shows the accuracy of the Qubit dsDNA HS assay compared to the accuracy of the Quant-iT PicoGreen dsDNA assay.

Table 2. Results of the Quant-iT PicoGreen dsDNA assay when plotted on a straight line. The quantitation reactions were carried out in a 96-well plate (200 µL/well) and read in a standard fluorescence microplate reader. The analysis is based on four replicates. The "accuracy index" is the average deviation from theoretical (known) concentration, plus CV. For CV (precision), blue indicates CV <5%. For accuracy index, red indicates values >15% and blue indicates values <15%.

DNA (ng/mL)	DNA (ng/200 µL)	CV (%)	Avg. dev. (%)	Accuracy index (avg. dev. + CV)
0	0	4.03		
0.5	0.1	3.92	838.03	841.95
1	0.2	2.93	416.73	419.66
5	1	2.31	88.15	90.46
10	2	2.73	39.43	42.16
50	10	1.86	1.41	3.28
100	20	3.74	3.44	7.18
200	40	1.49	1.37	2.87
400	80	1.36	2.06	3.42
600	120	1.11	2.00	3.10
800	160	0.65	5.28	5.93
1,000	200	1.92	4.47	6.39

Table 3. Results of the Quant-iT PicoGreen dsDNA assay when plotted on the Qubit dsDNA HS assay's nonlinear curve. The data are the same as those used for Table 2. Note that the last 2 data points, 800 and 1,000 ng/mL (shaded light blue), are out of the range of the dsDNA HS assay. The "accuracy index" is the average deviation from theoretical (known) concentration, plus CV. For CV (precision), blue indicates CV <5%. For accuracy index, red indicates values >15% and blue indicates values <15%.

DNA (ng/mL)	DNA (ng/200 µL)	CV (%)	Avg. dev. (%)	Accuracy index (avg. dev. + CV)
0	0	9.31		
0.5	0.1	7.87	41.64	49.51
1	0.2	5.27	21.59	26.86
5	1	3.05	12.44	15.49
10	2	3.27	2.56	5.83
50	10	1.95	6.30	8.26
100	20	3.83	5.31	9.14
200	40	1.51	2.73	4.24
400	80	1.37	1.48	2.85
600	120	1.11	1.32	2.43
800	160	0.65	25.46	26.11
1,000	200	1.92	15.27	17.19

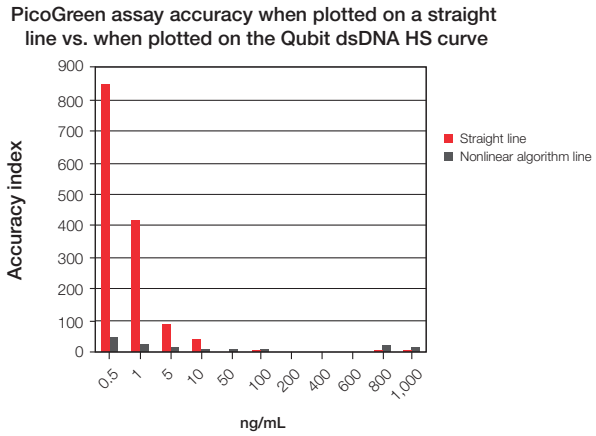
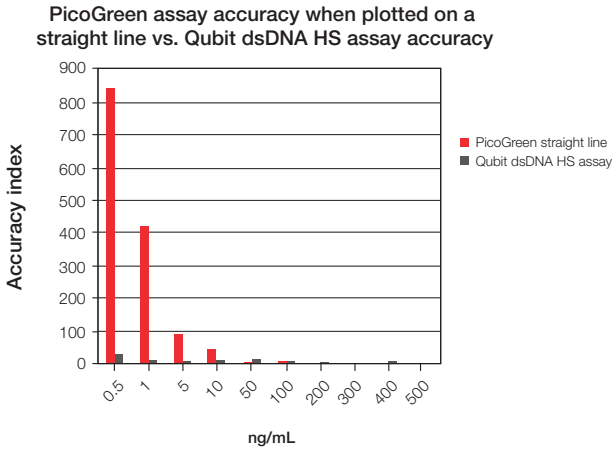


Figure 2. Accuracy of the Quant-iT PicoGreen assay when the same data are evaluated against a linear standard curve or the Qubit dsDNA HS nonlinear algorithm line. The accuracy index is defined in Table 2.



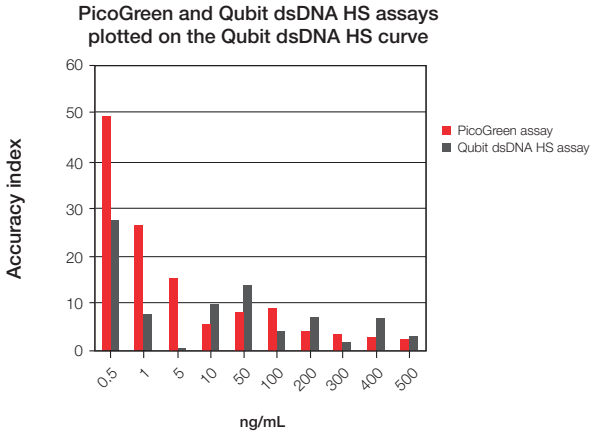


Figure 3. Accuracy of the Qubit dsDNA HS assay compared to the Quant-iT PicoGreen dsDNA assay plotted on two different types of curves. The accuracy index is defined in Table 2.

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Qubit dsDNA BR assay

The Qubit dsDNA BR assay was performed according to the kit instructions. Samples ranging in concentration from 0 ng/mL to 5,000 ng/mL were measured (10 replicates for each data point). Figure 4 shows the accuracy of the Qubit dsDNA BR assay compared to the accuracy of the Quant-iT PicoGreen dsDNA assay.

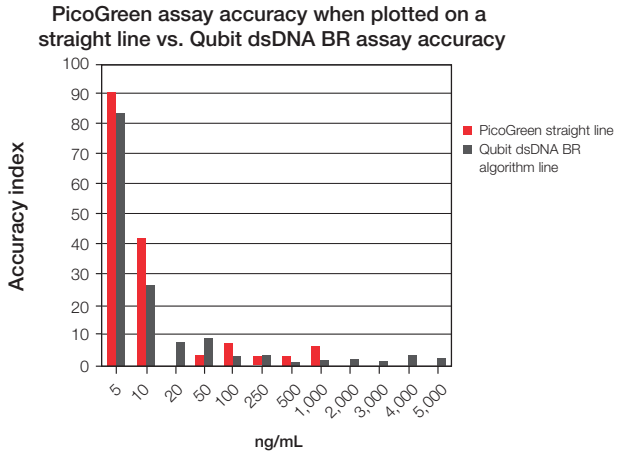

Figure 4. Accuracy of the Qubit dsDNA BR assay plotted on the algorithm line compared to the Quant-iT PicoGreen dsDNA assay plotted on a straight line. Note that this graph includes the middle and upper ranges of the PicoGreen assay, for which a linear standard curve gave good accuracy. The accuracy index is defined in Table 2.

Conclusions

The reported dynamic ranges and limits of detection for the Qubit dsDNA BR and HS assays (developed for the Qubit Fluorometer) are based on the accuracy of output related back to the standard-generated algorithm of the Qubit Fluorometer. For the Quant-iT PicoGreen dsDNA assay, results are based on aspects that include relative fluorescence units and Z′-factor scores.

While both assay types gave good accuracy over the middle and top of their ranges, the algorithms employed by the Qubit Fluorometer gave more accurate values over comparable full assay ranges because they are based on nonlinear curve fitting. The accuracy of the Quant-iT PicoGreen dsDNA assay depends on the accuracy of the curve generated by the user on the day of the assay using the standards provided in the kit. The data shown here for the Quant-iT PicoGreen assay were generated by plotting quantitation data on a straight-line standard curve. Using software packages such as GraphPad, researchers can easily fit standards to a curve (instead of a straight line), which should result in improved accuracy. For low-concentration samples, users can also create a low-range DNA standard curve according to the directions in the Quant-iT PicoGreen protocol, and plot the data on that curve.

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