Technical Overview

Agilent
Trusted Answers

Quantitative Analysis with the Agilent TapeStation Systems

Introduction

The Agilent TapeStation systems are automated electrophoresis instruments used for qualitative and quantitative analysis of DNA and RNA. The TapeStation portfolio consists of the Agilent 4150 TapeStation system for analysis of 1 to 16 samples at a time, while the Agilent 4200 TapeStation system is ideal for higher throughput laboratories with the ability to assess anywhere from 1 to 96 samples in a single run. The systems are important for sample quality control in a variety of workflows, such as next-generation sequencing. They are easily integrated into established workflows, with ScreenTape assays for different DNA and RNA applications that are fully compatible with each of the systems. The Agilent D1000 ScreenTape assays are ideal for analysis of DNA samples through 1,000 bp in length[1,2], while the Agilent D5000 ScreenTape assays are for longer DNA samples, with a sizing range from 100 to 5,000 bp[3,4]. Additionally, high sensitivity assays are available for samples of low abundance (Table 1). With these assays, the TapeStation systems provide a fast and easy way to assess sample sizing, quantification, and molarity.

Sizing and quantification analysis of DNA on the TapeStation has been compared to the Agilent 2100 Bioanalyzer system in previous publications[1,2,3,4]. Other nucleic acid quantification systems, such as the Thermo Fisher Scientific NanoDrop One^c and Thermo Fisher Scientific Qubit 4.0, use different technologies for DNA quantification, but do not provide sizing capabilities. In this technical overview, the quantitative performance of the TapeStation systems was systematically evaluated and compared to the Bioanalyzer system, NanoDrop, and Qubit.

Table 1. Sizing and quantitative specifications of the Agilent DNA ScreenTape assays for the Agilent 4150 TapeStation system and Agilent 4200 TapeStation system.

	D1000 ScreenTape	D5000 ScreenTape	High Sensitivity D1000 ScreenTape	High Sensitivity D5000 ScreenTape
Sizing Range	35 - 1,000 bp	100 - 5,000 bp	35 - 1,000 bp	100 - 5,000 bp
Sizing Accuracy	± 10%	± 10%	± 10%	± 15%
Sizing Precision	5% CV	5% CV	5% CV	10% CV
Quantitative Range	0.1 - 50 ng/µL	0.1 - 50 ng/µL	10 - 1,000 pg/µL	10 - 1,000 pg/µL
Quantitative Precision	0.1 - 1 ng/µL: 15% CV	0.1 - 1 ng/µL: 15% CV	15% CV	15% CV
	1 - 50 ng/µL: 10% CV	1 - 50 ng/µL: 10% CV		
Quantitative Accuracy	± 20%	± 20%	± 20%	± 25%

Experimental

The 4150 TapeStation and 4200 TapeStation systems were used in combination with the D1000 ScreenTape (p/n 5067-5582), D1000 reagents (p/n 5067-5583), High Sensitivity (HS) D1000 ScreenTape (p/n 5067-5584), and High Sensitivity D1000 reagents (p/n 5067-5585) as well as the D5000 ScreenTape (p/n 5067-5588), D5000 reagents (p/n 5067-5589), High Sensitivity D5000 ScreenTape (p/n 5067-5592), and High Sensitivity D5000 reagents (p/n 5067-5593).

NoLimits DNA fragments of 500 bp (Thermo Fisher Scientific, p/n SM1641) and 3,000 bp (Thermo Fisher Scientific, p/n SM1711) in length were prepared in 1x TE at a concentration of 50 ng/µL for the D1000 and D5000 assays or 1,000 pg/µL for the respective high sensitivity assays. The concentrations were confirmed in triplicate with spectrophotometric analysis (NanoDrop). Each sample was then further diluted in TE to fit the quantitative ranges of the DNA assays (Table 1).

The 500 bp fragment was used for assessment of the D1000 and HS D1000 assays, while the 3,000 bp fragment was used for the D5000 and HS D5000 assays. Twelve replicates of each concentration were assessed on each TapeStation instrument (a 4150 TapeStation and two different 4200 TapeStation models) using the respective assay with three ScreenTape and buffer lots, for a total of 108 data points for each dilution.

Three replicates of each fragment were also measured on Bioanalyzer, NanoDrop, and Qubit systems for comparison. For the Bioanalyzer system, two lots of the DNA 1000 assay (Agilent Technologies, p/n 5097-1504) were used, and samples were tested within the quantitative range of the DNA 1000 assay, from 0.5 to 50 ng/µL. For the NanoDrop, the highest concentration was measured, and the subsequent dilution concentrations were calculated based on nominal values. For the Qubit, two lots of the 1x dsDNA BR kit (Thermo Fisher Scientific, p/n Q33265) were used for comparison to the D1000 and D5000 ScreenTape assays, and two lots of the 1x dsDNA HS kit (Thermo Fisher Scientific, p/n Q33230) for comparison to the Agilent HS D1000 and HS D5000 ScreenTape assays. The measurements were repeated three times, such that the Bioanalyzer n=18, NanoDrop n=9, and Qubit n=18.

Results and discussion

Correlation of quantification measurements

A dilution series of a 500 bp DNA fragment was analyzed on the TapeStation systems using the D1000 and HS D1000 ScreenTape assays. Additionally, a 3,000 bp DNA fragment was assessed on the systems using the D5000 and HS D5000 ScreenTape assays. The TapeStation analysis software displays the results as both a digital gel and an electropherogram image. Illustrated in Figure 1 is an overlay of the electropherograms across the dilution series, highlighting the broad sizing and concentration ranges of the assays. The D1000 and HS D1000 assays have a sizing range from 35 to 1,000 bp, while the D5000 and HS D5000 assays extend from 100 through 5,000 bp. The D1000 and D5000 assays have a quantitative range from 0.1 to 50 ng/µL, while the quantitative range for the HS D1000 and HS D5000 assays is 10 to 1,000 pg/µL. The accuracy and precision specifications for the assays are shown in Table 1, and the sizing performance of the assays have been previously documented[1,2,3,4].

The dilution series of both fragments were quantified in multiple replicates to demonstrate the average quantitative performance of the D1000, HS D1000, D5000, and HS D5000 assays with the TapeStation systems. For comparison, the same samples were also assessed across multiple orthogonal systems for DNA quantification: the Bioanalyzer, NanoDrop, and Qubit. Each of the ScreenTape assays demonstrated very good linear correlations to all the systems tested, with R^2 values above 0.99 (Figure 2).

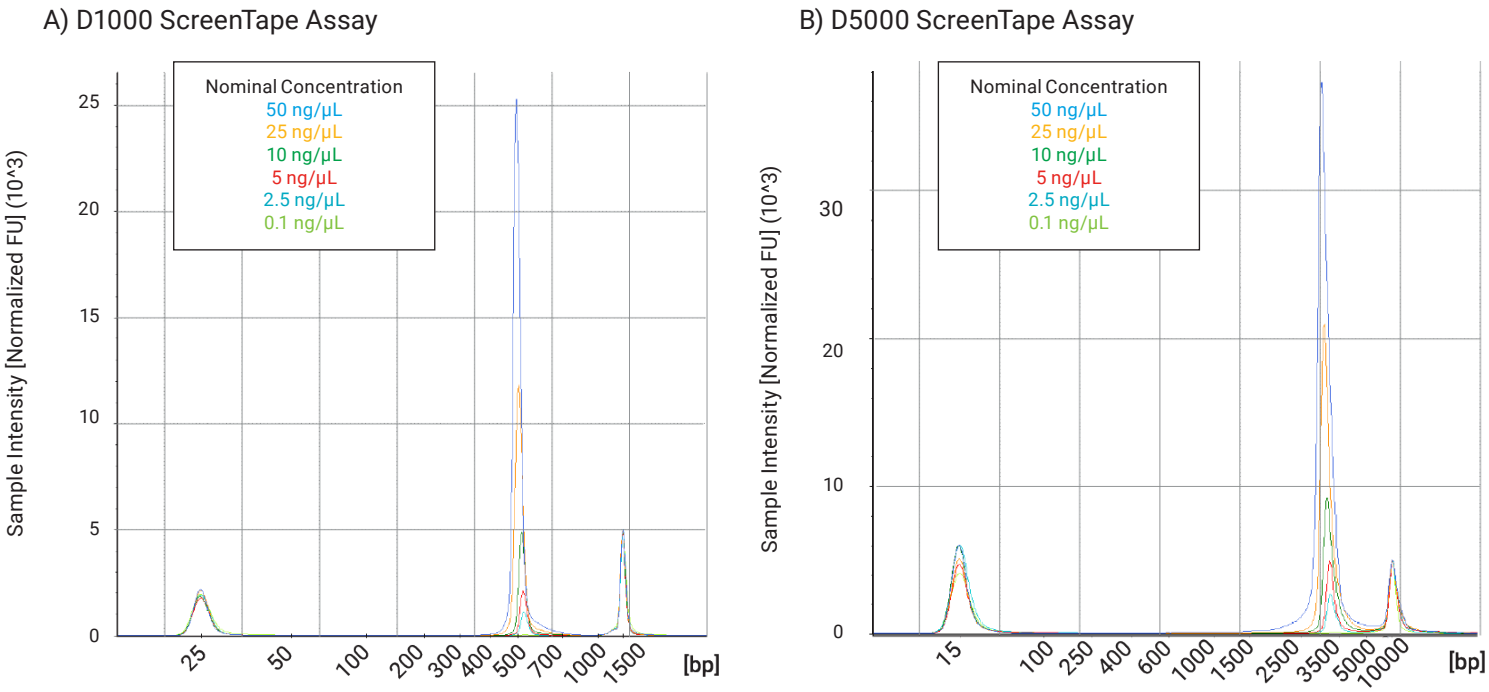

Figure 1. Serial dilutions of DNA fragments were analyzed on the Agilent TapeStation systems. Representative electropherogram overlays of A) a 500 bp fragment on the Agilent D1000 ScreenTape assay and B) a 3,000 bp fragment on the Agilent D5000 ScreenTape assay demonstrate the quantitative range of the assays, from 0.1 to 50 ng/µL.

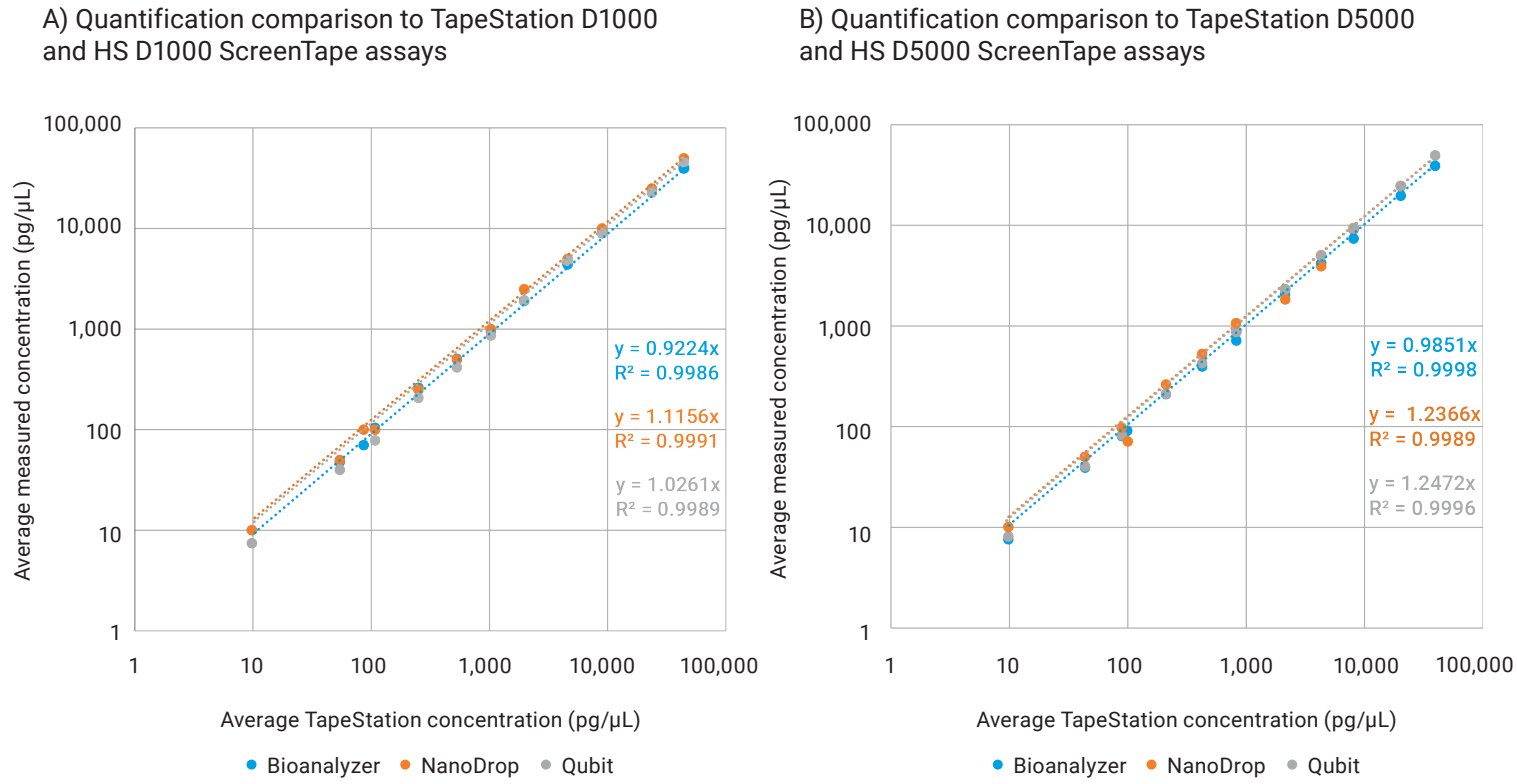

Figure 2. Quantitative comparison of the Agilent TapeStation systems to orthogonal systems, the Agilent 2100 Bioanalyzer system, the NanoDrop, and the Qubit. Samples were analyzed on the TapeStation systems using A) the Agilent D1000 and Agilent HS D1000 ScreenTape assays with a 500 bp DNA fragment, and B) the D5000 and HS D5000 ScreenTape assays with a 3,000 bp fragment.

Quantification accuracy

When assessing the accuracy of an instrument, it is necessary to compare the tested values to those of a known reference system. For quantification of nucleic acids, many orthogonal methods exist, each of which have their own specifications and limits of quantification. This inherent variability can therefore impact any accuracy calculations made in comparison to other instruments and should be taken into account when choosing a quantification system. To assess the quantification accuracy of the DNA ScreenTape assays, the TapeStation systems were compared to the Bioanalyzer, NanoDrop, and Qubit.

For the D1000 ScreenTape assays, the specification for quantification accuracy ±20%, was met in comparison to each of the other systems tested. The percent error of each sample across the quantitative range of the kit was calculated and the averages are shown in Figure 3. When taken together, the average percent error amongst all concentrations was 5.2% compared to the Bioanalyzer system, 10.2% compared to the NanoDrop, and 1.5% compared to Qubit.

Average quantification accuracy of the HS D1000 assay along the entire dilution series of the 500 bp fragment was successfully demonstrated against both the NanoDrop and Bioanalyzer system. The HS D5000 assay achieved accurate quantification for the 3,000 bp fragment dilution series compared to the Nanodrop and Qubit but did not meet expectations for the lower limit of quantification compared to the Bioanalyzer system. The D5000 ScreenTape assay demonstrated accuracy with all three of the other quantification systems, with limitations in the accuracy of the upper end of the concentration range tested in comparison to the NanoDrop and Qubit (data not shown). While a subset of samples did not match the quantification accuracy specification in comparison to the orthogonal instruments, it should be noted that the samples that did not pass were at the upper and lower quantitative ranges of the kits, and for best quantitative practices, samples should be assessed in the mid-range concentrations of the kits. Still, quantification accuracy for each of the DNA ScreenTape assays consistently aligned with at least one of the orthogonal quantification systems.

Quantification precision

Precision of quantification measurements was assessed for each of the ScreenTape assays on multiple TapeStation instruments. The precision for each concentration was well within the specifications of each assay, as shown in Figure 4. For example, the D1000 and D5000 assays state a precision specification of < 15% CV at 0.1 to 1 ng/µL and 10% CV for samples at 1 to 50 ng/µL. When analyzed with the D1000 assay, the 500 bp DNA fragment displayed excellent precision, with most of the concentrations at less than 3% CV. Similarly, the 3,000 bp fragment on the D5000 assay showed no more than an 8% CV for the samples within the 1 to 50 ng/µL range, and 11% for the lowest concentration samples at 0.1 ng/µL. The high sensitivity assays also displayed excellent precision, well below the specifications of 15% CV across the entire concentration range.

TapeStation instrument comparison

To further evaluate the quantitative capabilities of the TapeStation systems, the 4150 and 4200 instruments were compared. Shown in Figure 5 is the average measured concentration from assessment of the 500 bp fragment on both TapeStation models. Each concentration tested showed highly similar values, regardless of the TapeStation instrument used for analysis. For example, the 25 ng/µL sample had an average measured concentration of 23.31 ng/µL on the 4150 TapeStation and 23.86 ng/µL on the 4200 TapeStation. The D5000, HS D1000, and HS D5000 assays showed similar performances between the instruments (data not shown). Overall, the performance of each of the TapeStation models is equivalent to one another.

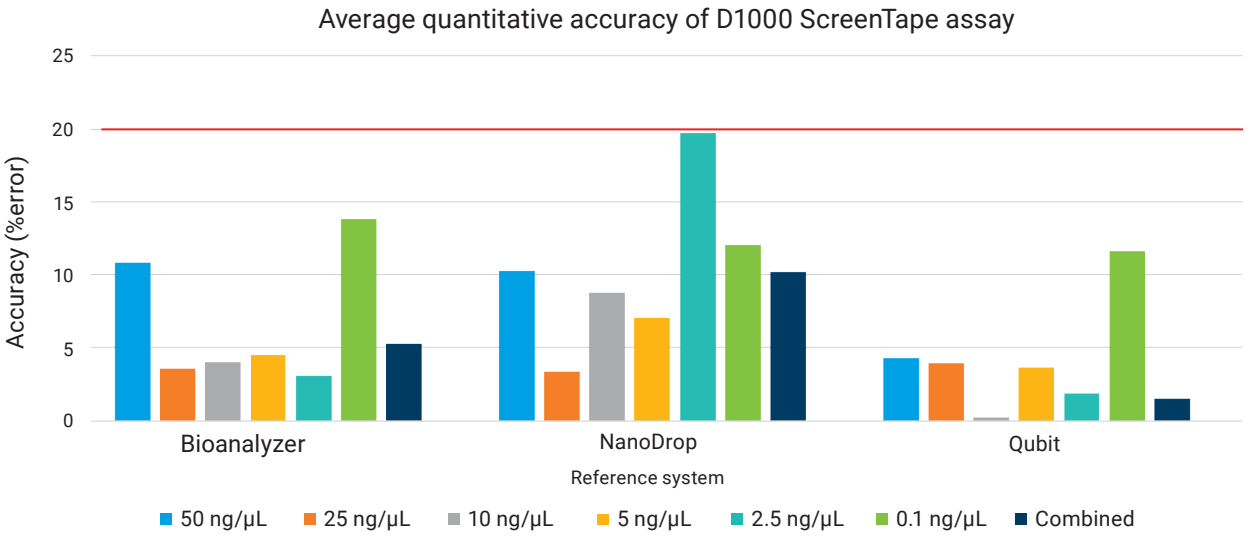

Figure 3. Average quantitative accuracy of the Agilent TapeStation systems (n = 108) with the Agilent D1000 ScreenTape assay compared to orthogonal quantification systems. Testing was performed using a dilution series of a 500 bp fragment, from 50 to 0.1 ng/µL. The specification for quantitative accuracy on the TapeStation systems was met for all systems and sample concentrations. The red line denotes the kit specification of 20% accuracy for the TapeStation D1000 ScreenTape assay. Note: the 0.1 ng/µL concentration is below the quantification specifications of the Bioanalyzer and Qubit assays.

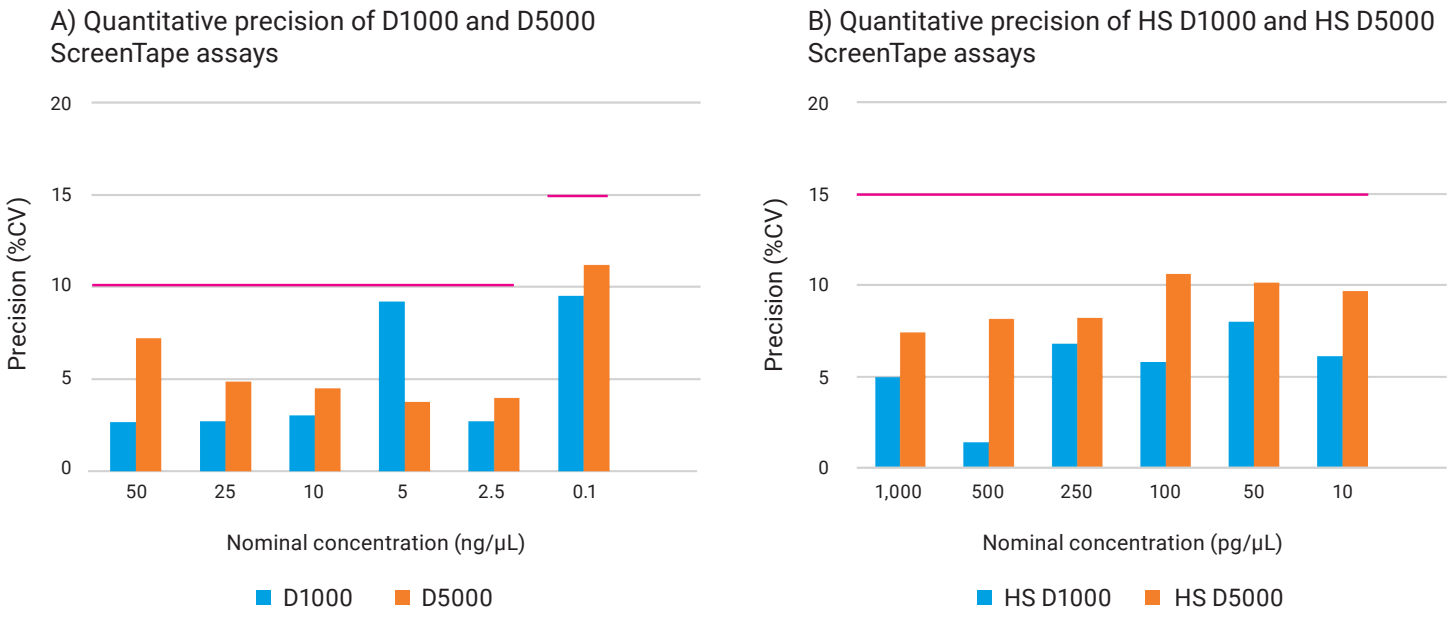

Figure 4. Average quantitative precision of the Agilent TapeStation systems with A) the Agilent D1000 and D5000 ScreenTape assays or B) the Agilent HS D1000 and HS D5000 ScreenTape assays. Assessment of the D1000 and HS D1000 assays was performed using a dilution series of a 500 bp DNA fragment. The D5000 and HS D5000 were assessed using a dilution series of a 3,000 bp fragment. The red line denotes the kit specification for quantitative precision using the TapeStation systems.

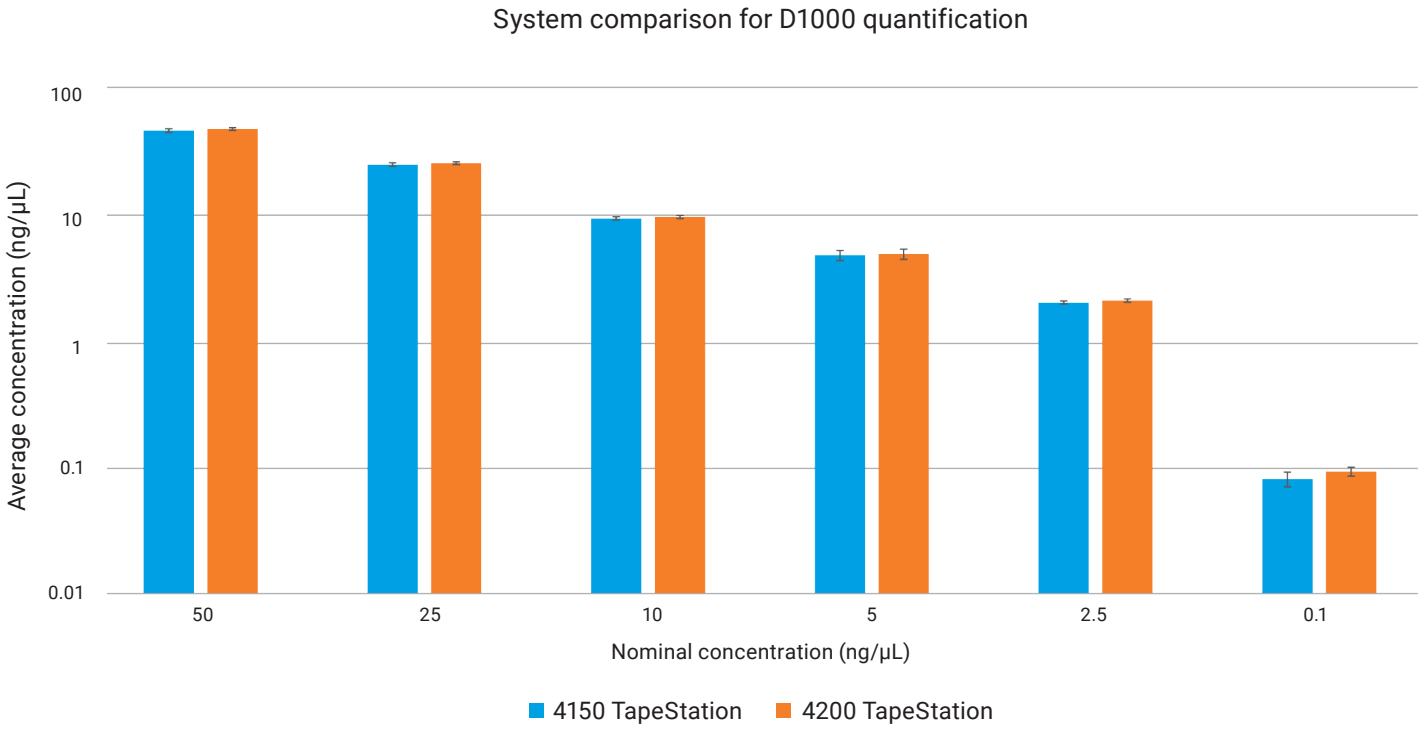

Figure 5. Equivalent performance of the Agilent 4150 and 4200 TapeStation systems is demonstrated through the quantification of a dilution series of a 500 bp DNA fragment using the Agilent D1000 ScreenTape assay. Error bars represent standard deviation.

Conclusion

This technical overview focuses on the quantitative analysis of DNA fragments assessed with Agilent TapeStation systems and Agilent ScreenTape assays, and provides a comparison to orthogonal systems for DNA quantification with the Agilent Bioanalyzer system, the NanoDrop, and the Qubit.

Quantification accuracy was evaluated by comparing the average measured values from the TapeStation systems to those of orthogonal systems. The specifications for quantification accuracy with the D1000 assay were met in comparison to each of the systems tested. For the D5000, HS D1000, and HS D5000 assays, quantification accuracy consistently aligned with at least one of the other systems. Instances where the accuracy did not align with the other systems most frequently occurred at the upper and lower limits of quantification of the assays. For best quantification results, it is recommended to avoid testing samples at the upper and lower limits of the quantification ranges. Additionally, there is inherent variation between any system used for quantification, each with their own specifications and limits of quantification, potentially impacting comparisons between instruments. For workflows that require quantification using a reference system, it is important to validate the methods to be used for specific samples when deciding which system to use.

The quantification performance of the TapeStation systems is indicated by strong linear correlations to each of the orthogonal systems tested, as well as the excellent precision achieved across all four ScreenTape assays tested. Additionally, the data provides a high correlation between the standard and high sensitivity assays and highlights equivalent performance of the two different TapeStation models for DNA quantification.

References

1. Performance Equivalence of the D1000 ScreenTape Assays on the Agilent TapeStation Systems. *Agilent Technologies Technical Overview*, publication number 5994-3114EN, **2021**.
2. Performance Characteristics of the D1000 and High Sensitivity D1000 ScreenTape Assays for the 4150 TapeStation System. *Agilent Technologies Technical Overview*, publication number 5994-0277EN, **2018**.
3. Performance Characteristics of the D5000 and High Sensitivity D5000 ScreenTape Assays for the 4150 TapeStation System. *Agilent Technologies Technical Overview*, publication number 5994-0679EN, **2019**.
4. Performance of the Agilent D5000 and High Sensitivity D5000 ScreenTape Assays for the Agilent 4200 TapeStation System. *Agilent Technologies Technical Overview*, publication number 5991-6356EN, **2015**.

www.agilent.com/genomics/tapestation

PR7001-3658

Published in the USA, February 1, 2025
5994-8050EN

Agilent
Trusted Answers