Technical Overview

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Performance Equivalence of the RNA ScreenTape Assays on the Agilent TapeStation Systems

Introduction

Agilent TapeStation systems are well-recognized, fully automated platforms that provide fast, scalable, and reliable electrophoretic analysis of nucleic acids. The RNA ScreenTape portfolio for TapeStation systems allows for efficient and consistent quantification of RNA samples, ensuring high sensitivity down to 100 pg/µL. The RNA assays compatible with eukaryotic and prokaryotic sample types also deliver essential information about sample integrity. The RNA integrity number equivalent (RIN^e) of the RNA ScreenTape assays is an objective quality score, developed specifically for TapeStation systems to evaluate the level of RNA degradation. The RIN^e, together with accurate quantitative capabilities enabled by the TapeStation system, provides researchers with a valuable tool for sample quality control in a variety of downstream applications. These include quantitative reverse transcription PCR (qRT-PCR), microarray studies, and next-generation sequencing (NGS).

In early 2021, Agilent introduced a new model of the Agilent 4200 TapeStation instrument. This technology update is aimed at bringing continued improvements and benefits to users of the platform. One of the main new feature is the option of self-maintenance, supported by a modified design of the ScreenTape nest. Users can now easily exchange the electrode cartridge, resulting in an overall optimized cost of ownership. In addition, a modified instrument optical system allows for a better match with application-specific fluorescent dyes. The new 4200 TapeStation system remains fully compatible with all existing ScreenTape applications and plastic consumables established for the legacy platform.

This technical overview highlights the performance of the Agilent RNA ScreenTape assay and Agilent High Sensitivity RNA ScreenTape assay on the new 4200 TapeStation system. It describes sensitivity, quantification, and integrity characteristics of both assays, validated in direct comparison with the results delivered by the legacy Agilent 4200 TapeStation as well as the lower-throughput Agilent 4150 TapeStation instruments.

Analytical specifications

Table 1 summarizes analytical specifications[1] for the RNA and High Sensitivity RNA ScreenTape assays for all three TapeStation models.

Experimental

Materials

The 4150 TapeStation (p/n G2992AA), legacy 4200 TapeStation (p/n G2991AA), and new 4200 TapeStation (p/n G2991BA) systems with the RNA ScreenTape (p/n 5067-5576), Agilent RNA ScreenTape sample buffer (p/n 5077), Agilent RNA ScreenTape ladder (p/n 5067-5578), High Sensitivity RNA ScreenTape (p/n 5067 5579), Agilent High Sensitivity RNA ScreenTape sample buffer (p/n 5067 5580), Agilent High Sensitivity RNA ScreenTape ladder (p/n 5067-5581), and the Agilent Rat Kidney Total RNA (p/n 737007) were obtained from Agilent Technologies, Inc. The Qubit 2.0 Fluorometer (p/n Q32866) with Qubit RNA HS Assay Kit (p/n Q32852) as well as the Qubit RNA BR (p/n Q10210) were acquired from Thermo Fisher Scientific Inc.

Sample preparation

A degradation series was prepared by incubating high-quality total RNA at 94 °C in a thermoblock for specified time intervals. For integrity analysis, three different degradation levels were obtained for each RNA assay. Intact total RNA was diluted with TE buffer to provide four concentrations within the entire quantitative range of both RNA and High Sensitivity RNA ScreenTape assays. These dilution series were utilized for quantification and sensitivity assessments. The nominal concentrations of the RNA samples were determined using the Qubit fluorometer with the respective assays.

Table 1. Comparison of analytical specifications of the Agilent RNA ScreenTape assay and Agilent High Sensitivity RNA ScreenTape assay.

Analytical Specifications	Agilent RNA ScreenTape Assay	Agilent High Sensitivity RNA ScreenTape Assay
Quality Score	RIN^e	RIN^e
RIN^e Functional Range[1]	25 to 500 ng/µL	1,000 to 25,000 pg/µL
Quantitative Range[3]	25 to 500 ng/µL	500 to 10,000 pg/µL
Quantitative Precision[3]	10% CV	15% CV
Quantitative Accuracy[3]	±20%	±30%
Sensitivity[2]	5 ng/µL	100 pg/µL
Maximum Buffer Concentration in Sample	200 mM Tris 20 mM EDTA 50 mM NaCl	10 mM Tris 1 mM EDTA

[1] RIN^e - RNA integrity number equivalent
[2] Signal-to-noise > 3 (single peak)
[3] Applicable to eukaryotic total RNA as sample

RNA analysis

The RNA analysis was performed with both RNA and High Sensitivity RNA ScreenTape assays on all three TapeStation models, according to the instructions contained in the Agilent quick guides for TapeStation systems[2,3]. The total RNA samples were separated in replicates of nine for both assays on single legacy 4200 and 4150 TapeStation instruments, and on three different new 4200 TapeStation instruments. Analysis of data generated by all TapeStation models was conducted using Agilent TapeStation software 4.1.

Results and discussion

Total RNA integrity analysis

Compared to DNA, RNA is a less stable nucleic acid that is especially sensitive to degradation by nucleases. When analyzed by electrophoresis, loss of integrity for RNA can be monitored by a decrease in height or complete disappearance of the peak corresponding to the 28S ribosomal fragment. Quality control of input RNA samples with a reliable metric is an important step that contributes significantly to the success of subsequent RNA applications.

The RIN^e is a quality score that was specifically developed for TapeStation systems. The RIN^e is established as an analogue to the RNA integrity number (RIN), the widely recognized quality metric for the Agilent 2100 Bioanalyzer system. The equivalency between RIN^e and RIN was previously successfully demonstrated in several technical overviews (for instance, on the 2200[4,5] and 4150[6] TapeStation models). For prokaryotic and eukaryotic total RNA samples, the TapeStation analysis software automatically calculates the RIN^e and displays it as a numeric value, on a scale from 1 to 10. A high RIN^e value indicates highly intact RNA, whereas a low RIN^e value corresponds to strong degradation. Analysis of RNA using the RIN^e quality metric enables a fast, reproducible, and objective assessment of sample integrity. A wide functional range of RIN^e ranging from 1 to 500 ng/µL allows for accurate quality assessment suited to diverse experimental designs without the need for ancillary dilution.

Three rat kidney total RNA samples were analyzed at different degradation levels using the RNA ScreenTape assays to demonstrate RNA integrity assessment by the RIN^e quality metric on the new 4200 TapeStation instrument. The TapeStation analysis software delivers the integrity results using a table format, or as a gel image with RIN^e values under the individual gel lanes (Figure 1A). Color-coded flagging functionality supported by the software can be optionally set to simplify the visual inspection of results. As shown in the electropherogram overlays, the decrease in size of the peak corresponding to the 28S ribosomal fragment is indicative of the degree of total RNA degradation (Figure 1B).

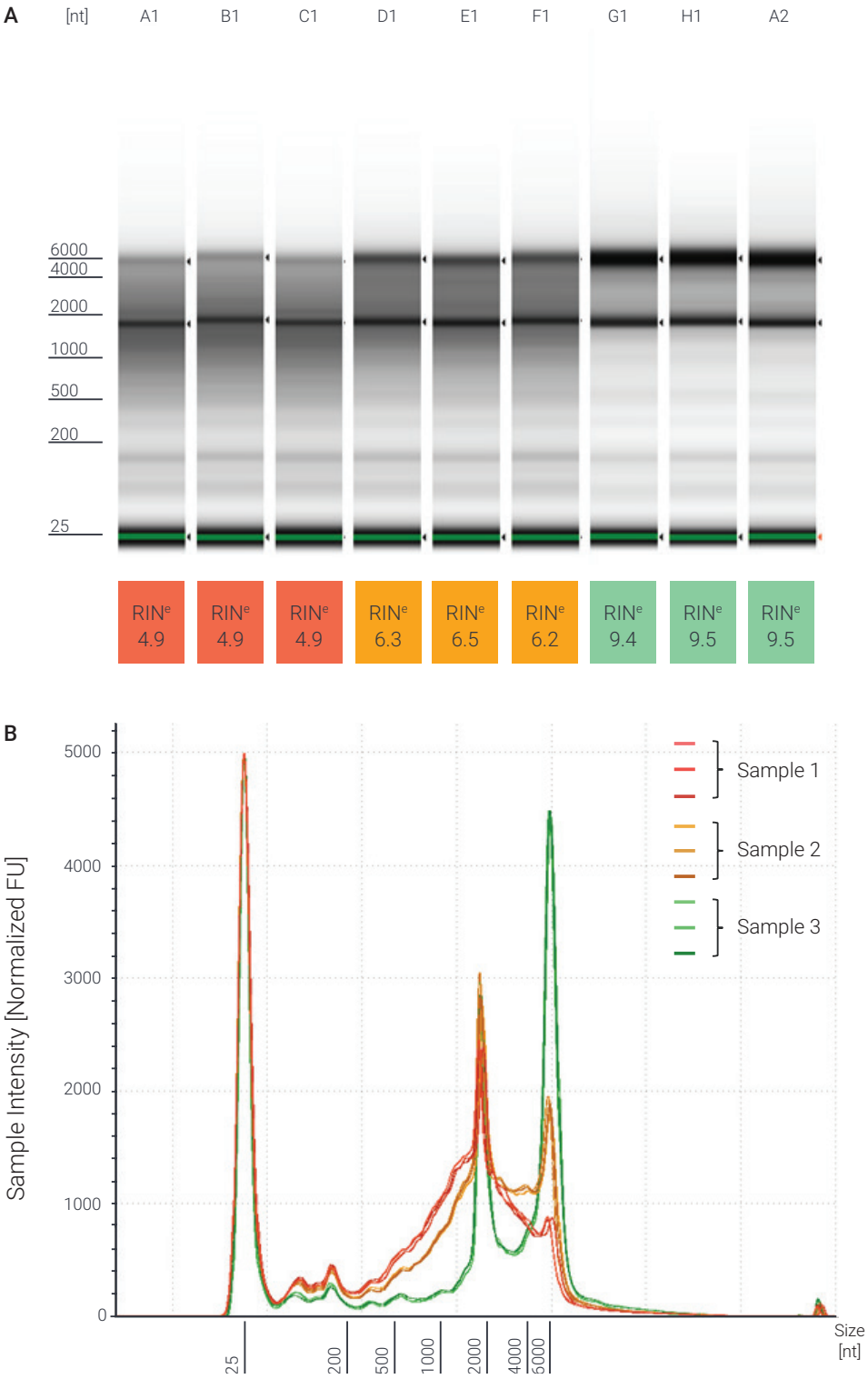

Figure 1. Three total RNA samples (50 ng/µL) with different levels of degradation were analyzed with the Agilent RNA ScreenTape assay on the new Agilent 4200 TapeStation instrument to assess RNA integrity using the RIN^e quality metric. The Agilent TapeStation analysis software delivers the results as an electropherogram or a gel image. (A) The RIN^e value is automatically determined, and directly displayed under the individual lane of the gel image. (B) The electropherogram overlay corresponding to the three RNA samples in triplicate.

The performance of total RNA integrity evaluation on the new 4200 TapeStation instrument was compared to the results generated by the other two models. The RIN^e numeric values obtained from all three TapeStation models were plotted pairwise for the RNA and High Sensitivity RNA ScreenTape assays. The scatterplots in Figure 2 show a high correlation between the new 4200 and the legacy 4200, as well as between the new 4200 and the 4150 TapeStation systems, with R^2 values of 99.9% to 100%.

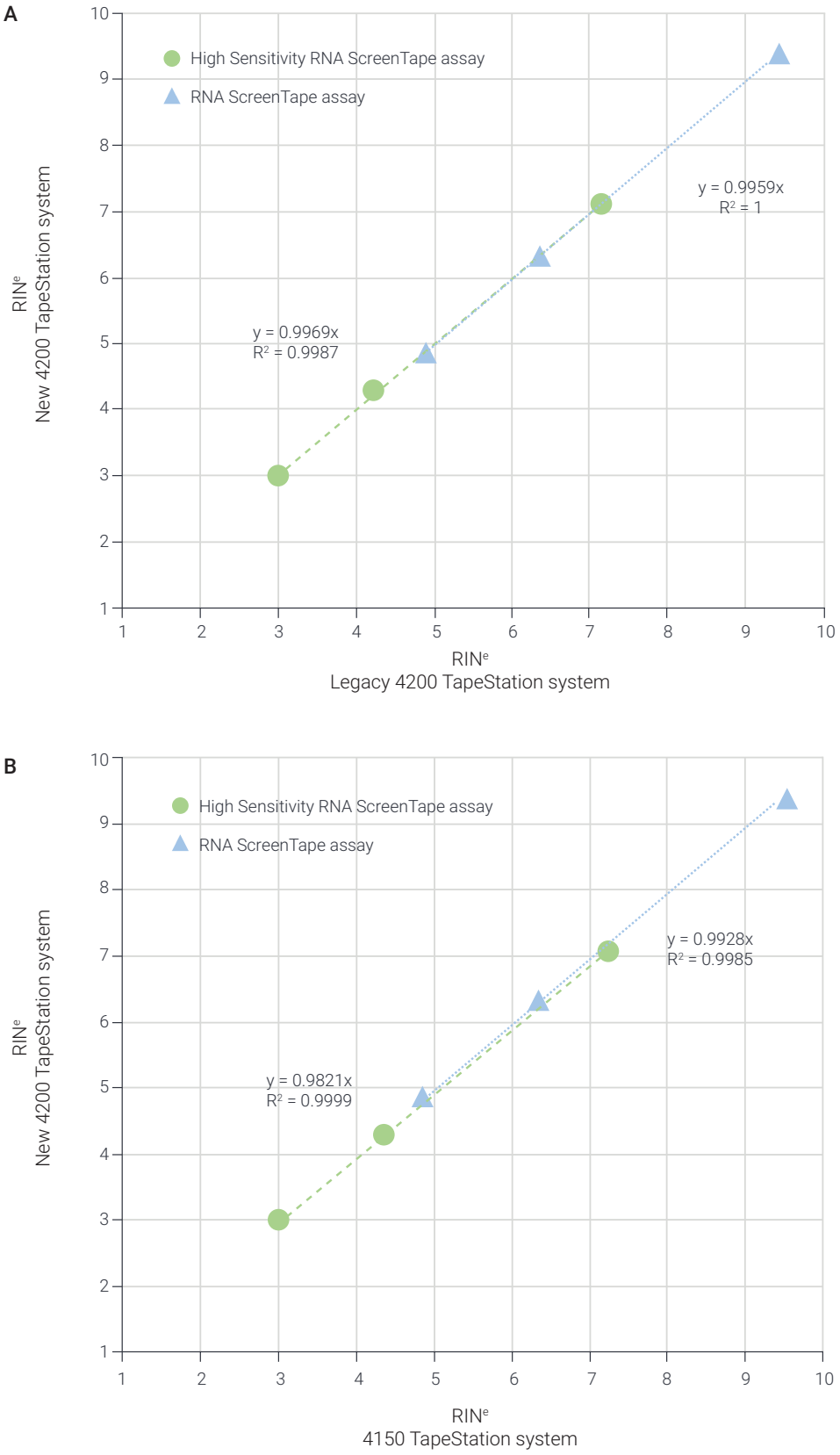

Figure 2. Correlation of RIN^e measurements generated by analysis of total RNA samples with varying degradation across three Agilent TapeStation models using both the Agilent RNA ScreenTape assay and Agilent High Sensitivity RNA ScreenTape assay. RIN^e results delivered by (A) the legacy Agilent 4200 TapeStation (X-axis) and (B) the Agilent 4150 TapeStation (X-axis) systems are plotted against the results obtained on the new Agilent 4200 TapeStation system (Y-axis).

In addition to equivalent RIN^e data across all three TapeStation models, the RIN^e quality metrics were found to be highly reproducible across the RNA degradation series. RIN^e precision was evaluated in terms of coefficient of variation (%CV) with nine replicates per total RNA sample for both RNA ScreenTape assays. As shown in Figure 3, highly precise results were obtained using RIN^e analysis with relative standard variation below 2.5% for the RNA and below 4% for the High Sensitivity RNA ScreenTape assay, respectively.

Overall, RIN^e quality metric results generated on the new 4200 TapeStation instrument were confirmed to be equivalent to the results delivered by the other two models. Moreover, high reliability and consistency of RIN^e measurements were demonstrated on different TapeStation models, including three new 4200 TapeStation instruments.

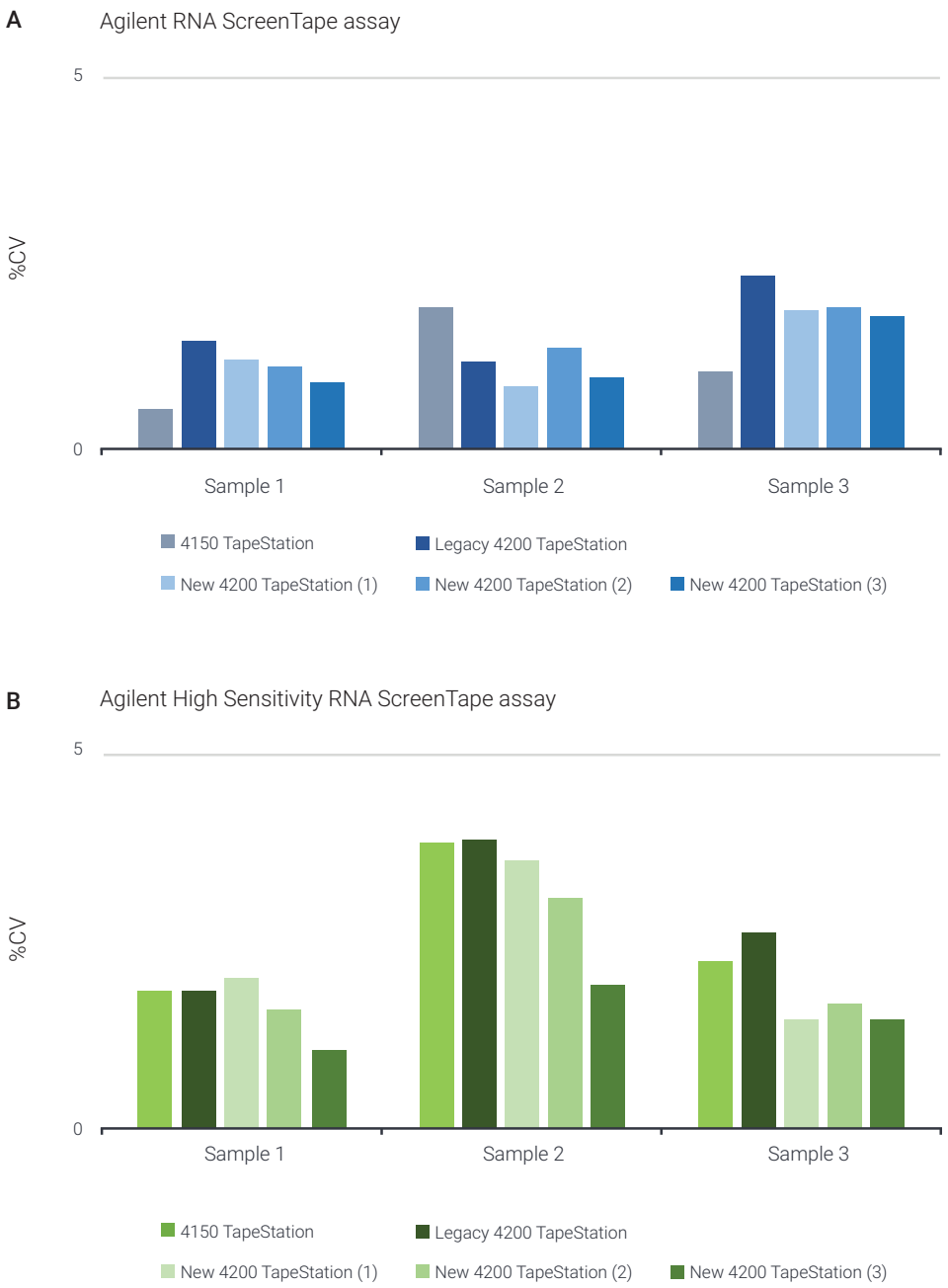

Figure 3. RIN^e precision determined on all Agilent TapeStation models (n = 9) across the total RNA degradation series using (A) the Agilent RNA ScreenTape assay and (B) the Agilent High Sensitivity RNA ScreenTape assay.

Sensitivity

Intact total rat kidney RNA samples at concentrations of 5 ng/µL and 100 pg/µL were analyzed on the new 4200 TapeStation system with both RNA ScreenTape assays. The electropherogram overlays of repeated analysis (n = 9) with enlarged sections are shown in Figure 4. The signal peaks of total RNA samples are clearly visible above the baseline at concentrations corresponding to the specified limits of detection (Table 1). Since the total RNA was detected with a signal-to-noise ratio (S/N) greater than three, the specified sensitivity of 5 ng/µL for RNA and 100 pg/µL for High Sensitivity ScreenTape assays was confirmed on the new 4200 TapeStation instrument.

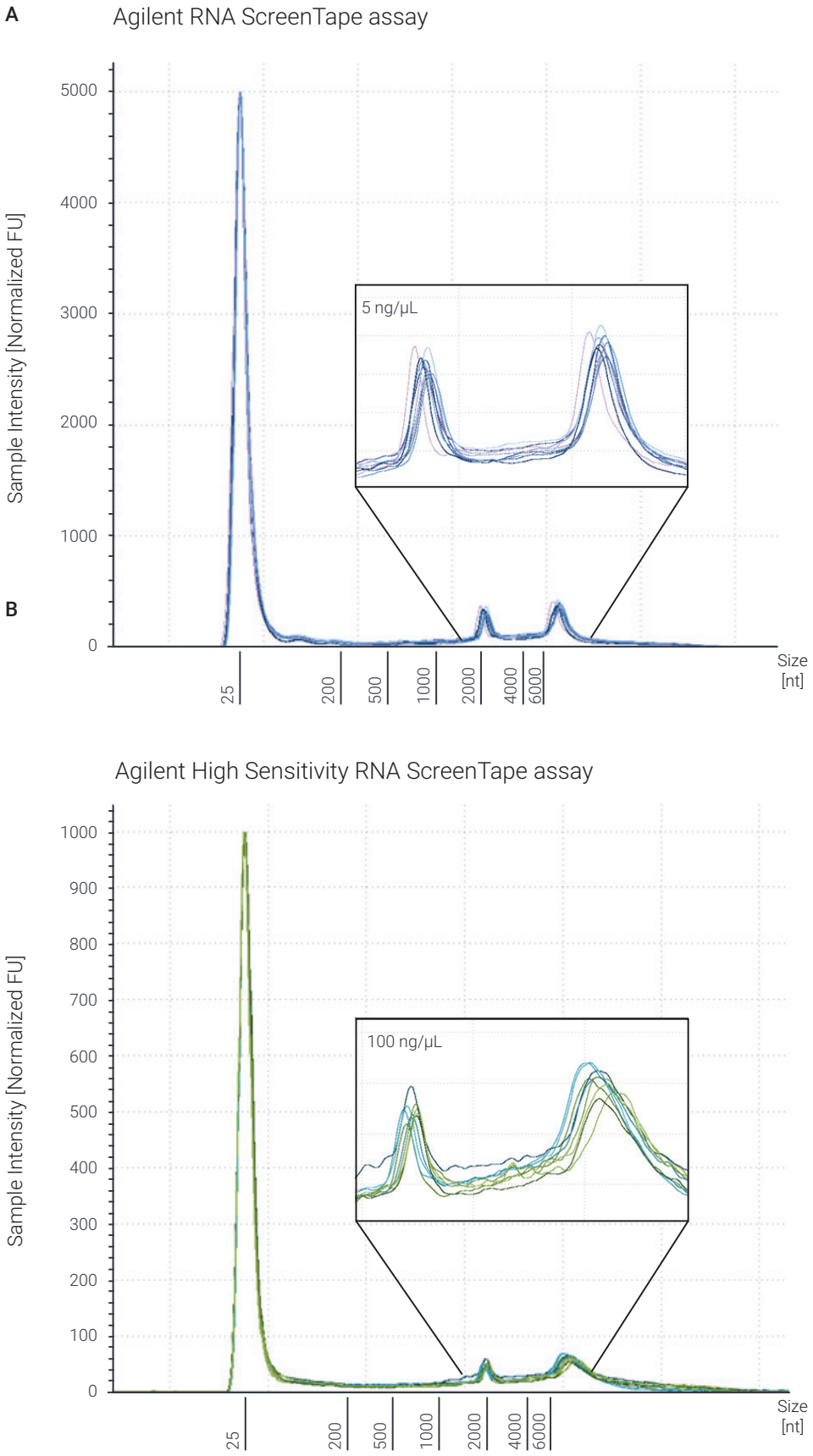

Figure 4. Electropherogram overlays in replicate (n = 9) of (A) 5 ng/µL RNA separated with the Agilent RNA ScreenTape assay, and (B) 100 pg/µL RNA separated with the Agilent High Sensitivity RNA ScreenTape assay using the new Agilent 4200 TapeStation system. The inset images show enlargement of the electropherograms.

Quantification

Detection and quantitation of nucleic acids are vital to many biological studies. The RNA ScreenTape analysis enables consistent and accurate results within quantitative ranges of 25 to 500 ng/µL for RNA, and from 500 to 10,000 pg/µL for High Sensitivity RNA ScreenTape assays, respectively. Quantitative accuracy and precision for both ScreenTape assays are presented in Table 1.

Serial dilutions of intact rat kidney total RNA covering the entire quantitative range of both RNA ScreenTape assays were analyzed on the new 4200 TapeStation system to evaluate the corresponding specifications. In addition, to demonstrate equal performance across all three TapeStation models, the same dilutions were utilized to assess concentrations on the legacy 4200 and 4150 TapeStation systems.

Direct comparison of the quantitative results between the new and the legacy 4200 TapeStation systems, as well as between the new 4200 and the 4150 TapeStation systems is shown in Figure 5. Comparative analysis of quantification data revealed excellent correlation, with R^2 values of 99.5% to 99.9% over all three TapeStation models. Quantitative accuracy of the new 4200 TapeStation system was well within the specifications for both RNA ScreenTape assays.

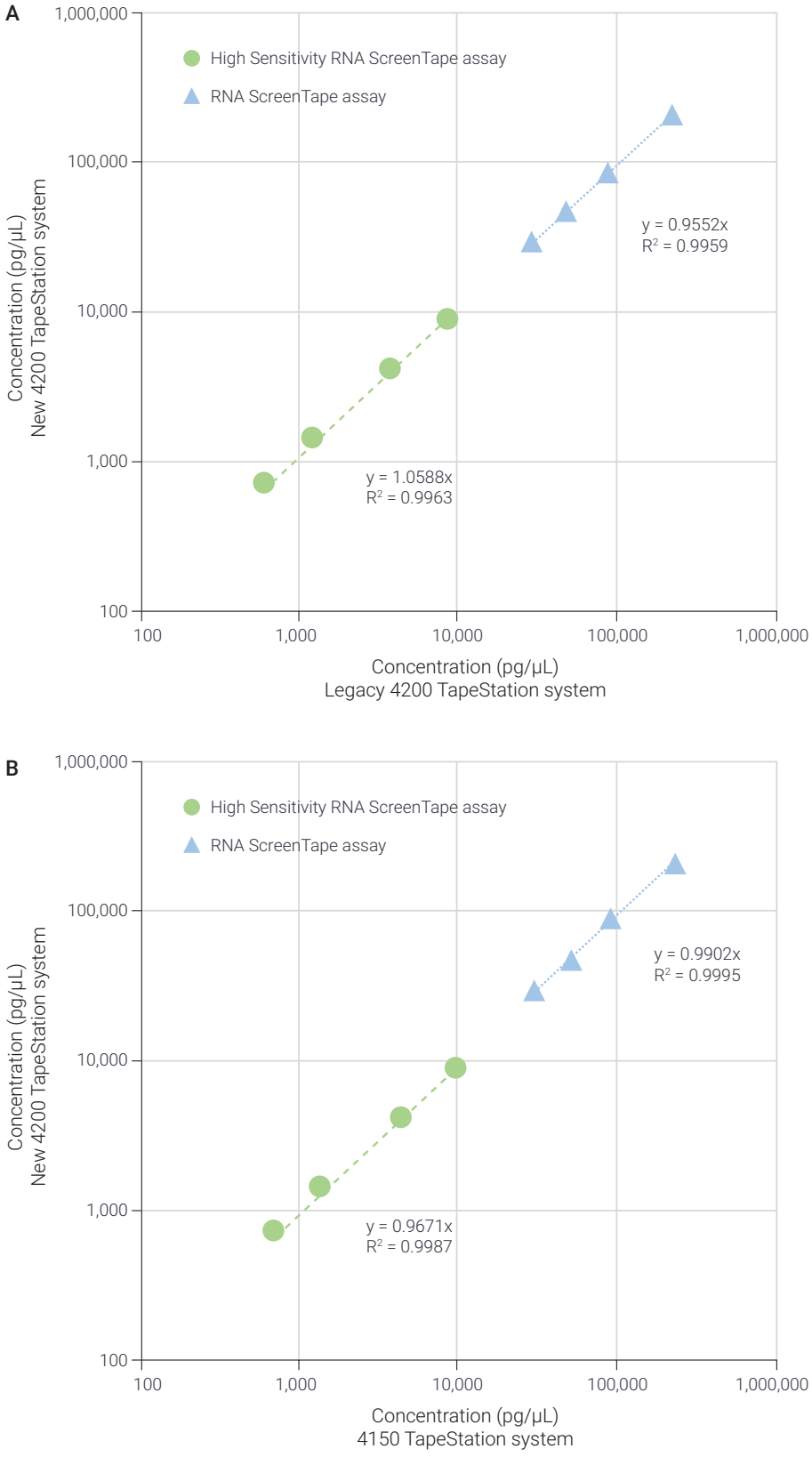

Figure 5. Correlation of quantitative data generated by the analysis of dilution series prepared from intact total RNA across three Agilent TapeStation models using both the Agilent RNA ScreenTape assay and Agilent High Sensitivity RNA ScreenTape assay. Concentrations determined on (A) the legacy Agilent 4200 TapeStation (X-axis) and (B) the Agilent 4150 TapeStation (X-axis) systems are plotted against the concentrations obtained on the new 4200 TapeStation system (Y-axis)

Figure 6 shows the precision of the quantification and the corresponding specifications displayed individually for each RNA ScreenTape assay. The quantitative precision was 7.6% or less for the RNA and 11.9% or less for the High Sensitivity RNA ScreenTape assay. Thus, the specified precision was met for both assays on all three TapeStation models.

Precise and accurate quantification performance of RNA and High Sensitivity RNA ScreenTape assays, previously systematically validated on the legacy 4200[7] as well as on the 4150[6] TapeStation systems, was also successfully verified on the new 4200 TapeStation system.

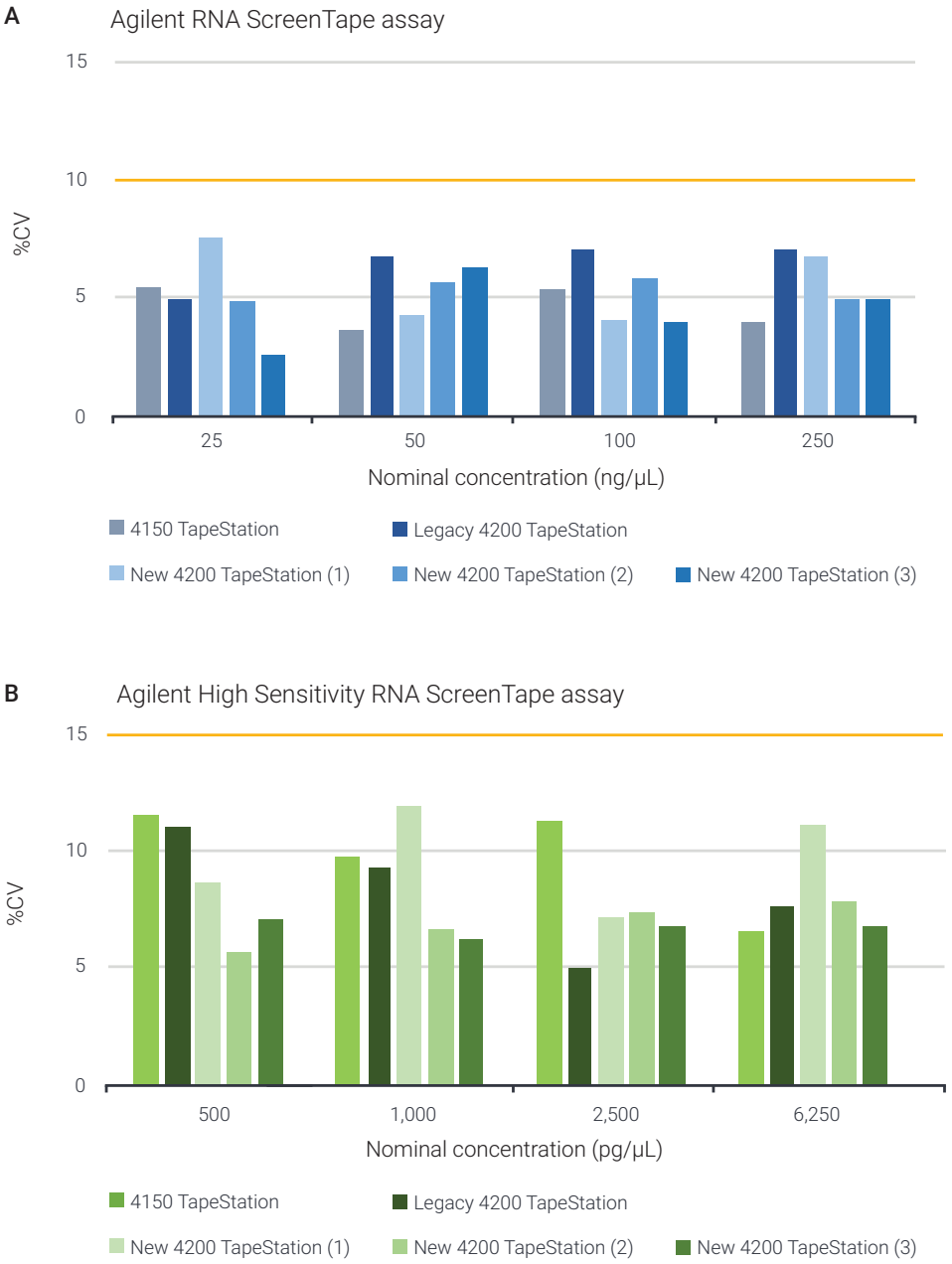

Figure 6. Quantitative precision assessed on all three Agilent TapeStation models (n = 9) across the total RNA serial dilutions using (A) the Agilent RNA ScreenTape assay and (B) the Agilent High Sensitivity RNA ScreenTape assays.

Conclusion

This technical overview demonstrates the excellent performance of Agilent RNA ScreenTape assays on the new Agilent 4200 TapeStation system verified by qualitative and quantitative RNA analysis. The analytical specifications for the Agilent RNA ScreenTape assay and the Agilent High Sensitivity RNA ScreenTape assay previously evaluated on the legacy 4200 TapeStation system were confirmed for the new 4200 TapeStation system. Furthermore, the data obtained with both RNA ScreenTape assays on the new 4200 TapeStation system were equivalent to those collected using the legacy 4200 and the Agilent 4150 TapeStation systems, ensuring full compatibility of the three models. Highly reproducible and concordant results delivered by all instruments supported the observance of high consistency between the models in relation to quantification and the RIN^e quality metric.

References

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