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Performance Comparison of the Experion™ Automated Electrophoresis System and a Competing Automated System for RNA Analysis

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Introduction

The assessment of RNA integrity and purity is an important first step to many gene expression analysis applications. It is preferable to use high-quality, intact RNA as a starting point for applications such as RT-PCR, ribonuclease protection assays, and northern analyses. For some applications, such as cDNA library construction and microarray analyses, which require considerable time and expense, it is essential to qualify the RNA sample before moving forward. Additionally, the stability of RNA transcripts can differ widely throughout the cell, and RNA degradation during extraction and handling can bias the quantitation of transcripts in gene expression studies. Therefore, a system that provides both sample quality analysis and accurate quantitation adds confidence and value to the results of downstream RNA-based applications.

The Experion automated electrophoresis system uses a combination of Caliper Life Sciences' innovative LabChip microfluidic separation technology and sensitive fluorescent sample detection to rapidly provide detailed information about RNA sample quality and concentration. Two Experion analysis kits are available for separation and detection of total RNA or mRNA at nanogram and picogram levels. The Experion RNA StdSens (standard-sensitivity) kit is designed for qualitative analysis and quantitation of 5–500 ng/µl total RNA and 25–250 ng/µl mRNA. The Experion RNA HighSens (high-sensitivity) kit is used for qualitative analysis of 100–5,000 pg/µl total RNA and 250–5,000 pg/µl mRNA samples. The Experion system combines the utility and qualitative benefits of gel-based RNA analysis with the quantitative accuracy of spectroscopic analysis.

The Agilent 2100 bioanalyzer is a related microfluidics-based electrophoresis system that uses LabChip technology and assays that are functionally similar to those used with the Experion system. In this report, we compare the performance of the Experion system and the bioanalyzer in analyzing both total RNA and mRNA samples. Included are a qualitative comparison of sensitivity for both systems and a comparison of the accuracy and reproducibility of RNA quantitation across the dynamic range for each system. The Experion system is demonstrated to provide improved RNA analysis by delivering increased sensitivity for better qualitative assessments and more reliable quantitation of RNA samples.

Methods

Rat brain total RNA and mRNA samples and the 6000 RNA ladder were purchased from Ambion, Inc. The Experion RNA ladder is a component of the Experion RNA StdSens and HighSens analysis kits, which were used for analysis using the Experion system. The RNA 6000 Nano and Pico LabChip kits were used with the Agilent 2100 bioanalyzer and were obtained from Agilent Technologies.

RNA samples and ladders were prepared according to the protocols described in the instruction manuals for the Experion RNA analysis kits and the Agilent 6000 LabChip kits. RNA samples and ladders were first heat-denatured for 2 min at 70°C and then kept on ice until use. The heat-denatured total RNA and mRNA stocks were diluted to the desired final concentrations in either RNase-free TE buffer, pH 7.0 (for use with the Experion RNA StdSens and RNA 6000 Nano LabChip analysis kits), or in DEPC-treated water (for use with the Experion RNA HighSens and RNA 6000 Pico LabChip analysis kits). RNA samples with concentrations ≥5 ng/µl were quantitated spectroscopically by measuring their absorbance at 260 nm, and these concentrations were used to evaluate the quantitation accuracy of both systems.

RNA chips were primed, loaded, vortexed, and analyzed according to the instructions provided with each analysis kit. Each concentration of total RNA or mRNA was analyzed on both systems using a minimum of five chips and three different wells per chip (n = 15 per concentration per RNA type, except for the lowest concentration tested, where n = 10).

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Results and Discussion

Qualitative Performance

To minimize any variation in labeling efficiency introduced by differences in sample preparation, both the Experion system and the bioanalyzer employ an RNA stain for detection that binds directly to the RNA moiety. Consequently, the fluorescence intensities of differently sized fragments depend only on the RNA sample concentration and sensitivity of the detection method. Thus, differences in the fluorescence intensity measurements displayed in an electropherogram translate directly into differences in sample concentration. The software in both the Experion system and in the bioanalyzer use the sample fluorescence intensity measurements to calculate quantitative information, such as RNA concentration.

To determine the sensitivity and range of detection of both systems, identical preparations of rat brain total RNA and mRNA at several concentrations were analyzed. The RNA StdSens and RNA 6000 Nano LabChip kits were compared using four concentrations of total RNA (5–500 ng/µl) and mRNA (25–250 ng/µl). The RNA HighSens and RNA 6000 Pico LabChip kits were compared using picogram levels of total RNA (100–5,000 pg/µl) and mRNA (250–5,000 pg/µl). Electropherograms were examined for qualitative differences between the two systems.
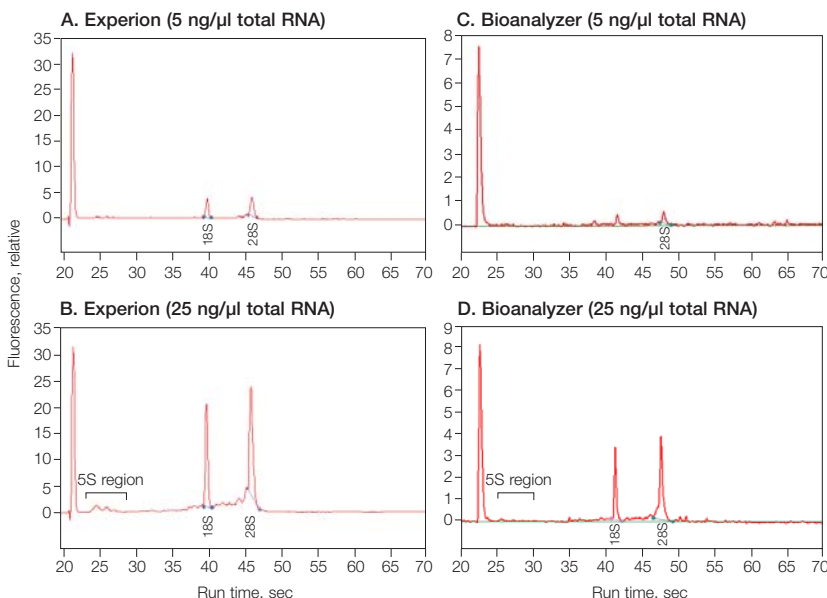
These analyses revealed three general trends. First, both the RNA HighSens and RNA 6000 Pico LabChip analyses correctly identified the ribosomal RNA peaks at all concentrations examined in the picogram concentration series (data not shown), but only the RNA StdSens analysis kit automatically identified the 18S ribosomal peak at the 5 ng/µl concentration of total RNA (Figure 1A and C) and 25 ng/µl concentration of mRNA (data not shown). Second, the fluorescence intensities generated by the 18S and 28S peaks in a given sample were, on average, 4–5 times higher on the Experion system than on the bioanalyzer (Figure 1).

The greater detection sensitivity of the Experion system allows the generation of a higher signal-to-noise ratio; consequently, the consumption of smaller amounts of RNA sample is possible. Third, certain details, such as the presence of small peaks in the 5S region (between the lower marker and 18S RNA) and in the region between the two large ribosomal RNAs, were better resolved above the baseline by the Experion system (Figure 1B and D), potentially leading to better assessment of degraded RNA species or contaminating genomic DNA. By providing additional sample information, such as the presence or absence of a peak or difference in the intensity of a fluorescent signal over the baseline in identical RNA samples, the Experion system demonstrated greater sensitivity than the bioanalyzer.

Quantitative Performance

In addition to providing a quick visual assessment of RNA quality and integrity, Experion software also performs sample quantitation (calculation of RNA concentration) and provides these results in a Results table. Knowing the sample quality and having accurate and reproducible quantitative data facilitates the successful planning and execution of downstream experiments, such as cDNA construction, microarray analysis, and RT-PCR analysis.

Experion software measures RNA concentration by calculating the area under the electropherogram of an RNA sample and comparing it to that of the RNA ladder, which is provided in the Experion RNA analysis kits at a known concentration. This approach generally yields accurate and reproducible results, though it should be noted that the quality of the RNA ladder preparation and the consistency of chip preparation are also important factors in determining overall quantitation performance. Because the Experion system and the bioanalyzer use similar methods for determining RNA sample concentrations, the accuracy and reproducibility of RNA quantitation with both systems may be directly compared.

A. Experion (5 ng/µl total RNA)
C. Bioanalyzer (5 ng/µl total RNA)
B. Experion (25 ng/µl total RNA)
D. Bioanalyzer (25 ng/µl total RNA)

Fig. 1. Comparison of sensitivity. Electropherograms were obtained from total RNA separations performed on the Experion system (A and B) and the Agilent 2100 bioanalyzer (C and D) using the nanogram analysis kits. The Experion system's greater sensitivity is shown by the higher fluorescent signal, which allowed improved sample detection. For 5 ng/µl total RNA, the 18S ribosomal RNA peak was automatically identified by the Experion system software (A), but not by the bioanalyzer software (C). For 25 ng/µl total RNA, the Experion system also resolved peaks in the 5S region, offering additional RNA sample information (B), while the bioanalyzer did not provide the same level of detail (D).

To determine accuracy, the concentration of each RNA sample was analyzed with both systems and was measured spectroscopically using UV absorbance at 260 nm. Accuracy, defined as the percent difference between the RNA concentration calculated by the Experion system or the bioanalyzer (chip measurement) and that derived spectroscopically, was determined using the formula: [(software concentration – UV concentration)/UV concentration] x 100. Values close to zero indicate parity between the chip and spectroscopic measurements, and a negative or positive value indicates an underestimation or overestimation, respectively, of the chip measurement relative to the spectroscopic measurement.

Reproducibility was evaluated using the coefficient of variation, or CV, as a statistical measure. For each RNA preparation tested, the CV of the concentration reported by the software was determined using the formula: [standard deviation/mean] x 100. CV was expressed as a percentage; small CV values indicate a small degree of variation in replicates and good quantitative reproducibility of the data.

The results of these experiments are summarized in Tables 1 and 2 according to the type of RNA, concentration of RNA, and analysis kit used. The data shown in these tables represent the average measurement of the interchip (across multiple chips) accuracy and reproducibility for a given concentration and type of RNA.

Overall, the Experion system provided more accurate RNA quantitation than the bioanalyzer in both the nanogram and picogram range and for both total RNA and mRNA samples. Whereas the RNA StdSens kit displayed a maximum deviation from spectroscopic measurements for total RNA samples of 13.7%, the RNA 6000 Nano LabChip kit produced up to 24.1% deviation for the same set of samples (Table 1). Similarly, the RNA StdSens kit produced measurements within 7.5% of spectroscopic values for mRNA, compared to a maximum 17.0% deviation produced by the RNA 6000 Nano LabChip kit (Table 1). The quantitation accuracy of the RNA HighSens and RNA 6000 Pico LabChip kits was also examined at a single concentration of total RNA and mRNA that could be measured by UV spectroscopy (5,000 pg/µl). The differences between the spectroscopically measured concentrations and those obtained using the RNA HighSens kit were 19.6% for total RNA and 6.8% for mRNA, compared to 67.9% and 81.7% using the RNA 6000 Pico LabChip kit (Table 2). These data demonstrate that the Experion system generally provides concentration measurements that are most similar to those obtained by spectroscopic methods.

The Experion system also displayed greater reproducibility than the bioanalyzer when used for RNA quantitation. With total RNA samples, interchip CVs for the RNA StdSens and HighSens kits ranged between 5.3 and 32.9%, while those for the RNA 6000 Nano and Pico LabChip kits ranged between 9.0 and 48.3%. Similarly, the interchip CVs for mRNA samples ranged from 4.9

Table 1. Comparison of accuracy* and reproducibility** of quantitation of nanogram levels of RNA. Total RNA and mRNA samples at the indicated concentrations were analyzed with the Experion RNA StdSens analysis kit and the RNA 6000 Nano LabChip kit using the Experion system or the Agilent 2100 bioanalyzer, respectively.

|	Experion		Bioanalyzer	
	Accuracy	Reproducibility	Accuracy	Reproducibility
Total RNA				
5 ng/µl	–10.7%	32.9%	21.6%	48.3%
25 ng/µl	–11.6%	11.6%	–24.1%	13.6%
100 ng/µl	–13.7%	5.3%	–20.6%	13.6%
500 ng/µl	–8.9%	7.9%	3.4%	9.0%
1,000 ng/µl***	3.4%	5.8%	—	—
mRNA				
25 ng/µl	7.5%	20.3%	17.0%	24.8%
50 ng/µl	0.4%	14.7%	–9.4%	33.0%
100 ng/µl	–4.6%	10.0%	–14.5%	30.0%
250 ng/µl	–1.7%	4.9%	10.0%	31.1%

*   Calculated as % difference relative to values determined by UV.
**   Calculated as % CV.
*** The 1,000 ng/µl standard is outside the published concentration range for both the Experion system and the bioanalyzer, so the bioanalyzer data are not included.

Table 2. Comparison of accuracy* and reproducibility** of quantitation of picogram levels of RNA. Total RNA and mRNA samples at the indicated concentrations were analyzed with the Experion RNA HighSens analysis kit and the RNA 6000 Pico LabChip kit using the Experion system or the Agilent 2100 bioanalyzer, respectively.

|	Experion		Bioanalyzer	
	Accuracy	Reproducibility	Accuracy	Reproducibility
Total RNA				
100 pg/µl***	—	9.1%	—	—
200 pg/µl	—	13.9%	—	17.0%
500 pg/µl	—	12.4%	—	10.2%
1,000 pg/µl	—	12.5%	—	26.1%
5,000 pg/µl	19.6%	9.4%	67.9%	9.4%
mRNA				
250 pg/µl***	—	17.6%	—	—
500 pg/µl	—	29.9%	—	19.7%
1,000 pg/µl	—	23.6%	—	19.1%
5,000 pg/µl	6.8%	33.4%	81.7%	22.4%

*   Calculated as % difference relative to values determined by UV.
**   Calculated as % CV.
*** The Experion system has a sensitivity range with a lower end as low as 100 pg/µl (total RNA) and 250 pg/µl (mRNA). The bioanalyzer specifications are 200 pg/µl (total RNA) and 500 pg/µl (mRNA).

to 33.4% and from 19.1 to 33.0% for the Experion system and the bioanalyzer, respectively (Tables 1 and 2). For both systems, reproducibility appeared to be higher for total RNA than for mRNA and tended to be the most reproducible at higher RNA concentrations. The total RNA and mRNA quantitation data generated by the RNA 6000 Pico LabChip kit appear to be similar to those generated by the RNA HighSens kit; however, the bioanalyzer consistently and significantly overestimated the concentration of 5,000 pg/µl RNA preparations, as indicated by the accuracy data for these experiments (Table 2).

Figures 2 and 3 provide graphic representations of the performance data in Tables 1 and 2 and both the interchip and intrachip (multiple wells within one chip) variations in accuracy and reproducibility. The distribution of data in these figures clearly illustrates that the chip-to-chip variations in both accuracy and reproducibility were, in general, smaller for the Experion system, regardless of the type of RNA analyzed.
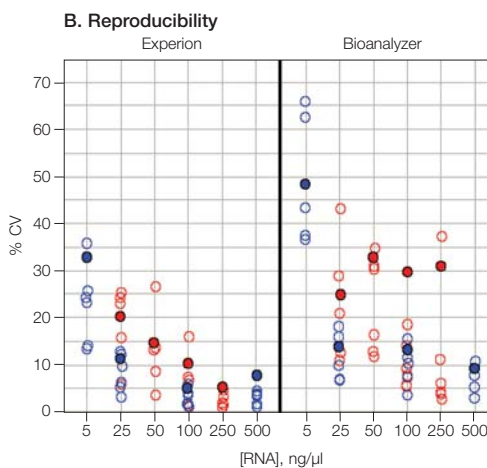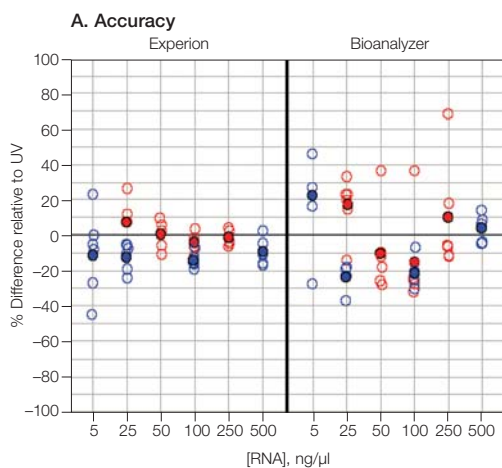
Fig. 2. Scatter plot comparison of quantitation accuracy (A) and reproducibility (B) data for nanogram levels of RNA. Graphical representation of intrachip data (○, mRNA; ○, total RNA; average of 3 wells/chip) and interchip data (●, mRNA; ●, total RNA; average of 5 chips), also presented in Table 1. Left panels, data generated by the Experion RNA StdSens analysis kit; right panels, data generated by the RNA 6000 Nano LabChip kit.
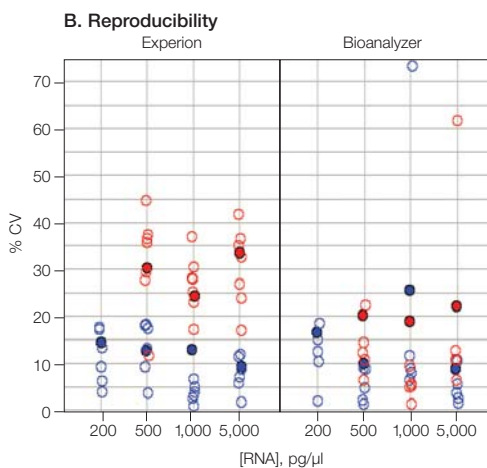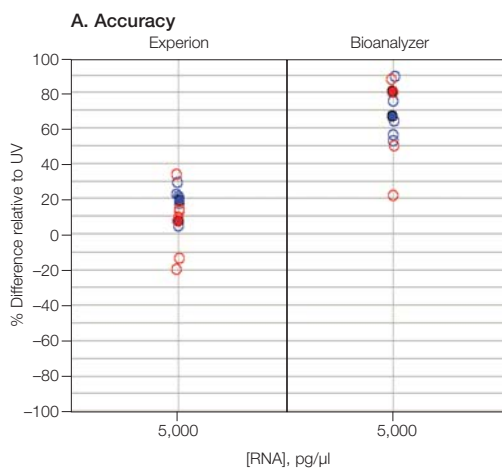
Fig. 3. Scatter plot comparison of quantitation accuracy (A) and reproducibility (B) data for picogram levels of RNA. Graphical representation of intrachip data (○, mRNA; ○, total RNA; average of 3 wells/chip) and interchip data (●, mRNA; ●, total RNA; average of 5 chips), also presented in Table 2. Left panels, data generated by the Experion RNA HighSens analysis kit; right panels, data generated by the RNA 6000 Pico LabChip kit.

Conclusions

The Experion system's greater sensitivity of detection provides the capability to automatically acquire additional sample information, such as the presence or absence of a peak, or peak variations in identical samples, for better qualitative assessment of RNA samples. Overall, the Experion system provides RNA concentration measurements that are more accurate, as they are most similar to those obtained by the traditional spectroscopic methods. The Experion system also displays better reproducibility in RNA sample quantitation, as illustrated by the observed small variations in both interchip and intrachip CV values. The Experion system combines two valuable sample assessments, qualitative and quantitative, into a single analytical process, and provides higher sensitivity, better quantitation accuracy, and greater reproducibility for both total RNA and mRNA samples.

For more information on the Experion system, see bulletins 5285 and 3174A.

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