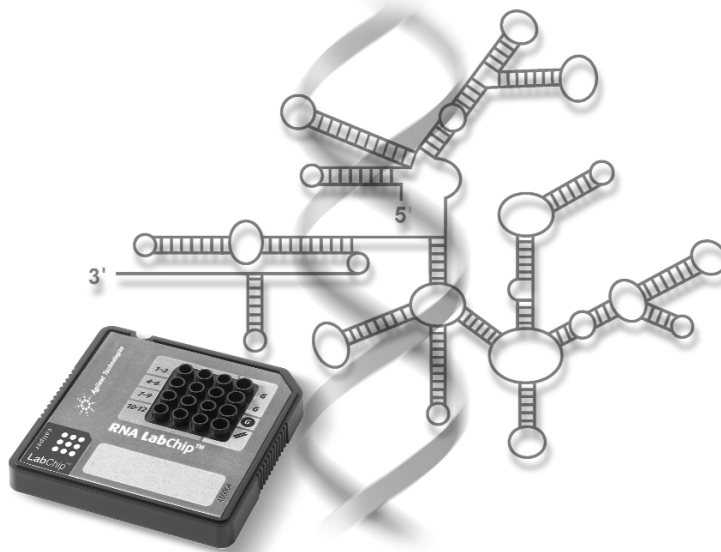# Analysis of total RNA using the Agilent 2100 Bioanalyzer and the RNA 6000 LabChip® kit

Application Note

Meike Kuschel

Abstract

The RNA 6000 LabChip® kit can be used with the Agilent Technologies 2100 bioanalyzer to determine the integrity and measure the concentration of total RNA samples obtained from various mouse tissues and from *E. coli*. For each sample the data analysis software automatically calculated the ratio of ribosomal RNA peaks and reported the total RNA concentration in ng/µl. The resolution of the RNA separations on the chip was found to be as good or better than that achieved using denaturing agarose gels and the quantitation was comparable to UV absorption measurements.

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Introduction

Isolation of pure and intact RNA is essential for many applications. Depending on the purification process, the quality and integrity of total RNA preparations can vary significantly. Applications for which this information is useful include RT-PCR, preparation of targets for microarrays, ribonuclease protection assays, preparation of cDNA libraries, and Northern blotting among others.

To examine the quality of total RNA preparations, denaturing agarose gel electrophoresis followed by ethidium bromide staining is the most commonly used method. The separation is usually done under denaturing conditions to exclude the effect of altered mobility due to the RNA secondary structures. However, for accurate analysis, this traditional technology typically requires significant amounts of sample and an additional method for quantitation. RNA concentration is typically determined by measuring the absorbance at 260 nm in a spectrophotometer. The ratio between the absorbance at 260 and 280 nm gives a rough estimate of RNA purity with respect to contaminants that absorb at a different wavelength, such as proteins, but provides no information regarding possible degradation of the RNA by nucleases during the purification process.

With the increased interest in expression profiling applications, there has been a steadily growing demand for faster, more automated analysis tools that consume minimal sample amounts. Lab-on-a-Chip technology[1-5] is particularly well suited for the rapid analysis of nucleic acids because it integrates multiple experimental steps. With the introduction of the Agilent 2100 bioanalyzer utilizing LabChip technology from Caliper Technologies Corp., sample handling, separation, and analysis have been integrated in a more automated manner. Chip-based analysis offers several benefits over existing technology including reduced sample consumption, minimized manual intervention, increased analysis speed and data precision, and minimized exposure to hazardous materials.

The RNA 6000 LabChip kit can be used with the Agilent 2100 bioanalyzer to measure both the quantity and the integrity/purity of RNA samples. The integration of these two measurements provides a simple and quick assay that consumes minimal sample amounts.

Materials and methods

Agilent 2100 bioanalyzer instrument and software

All chip-based separations were performed on the Agilent 2100 bioanalyzer, which was controlled by dedicated software running on a PC. The Agilent 2100 biosizing software includes data collection, presentation and interpretation functions. Data can be displayed as a gel-like image and/or as electropherogram(s). For each sample, the ratio of the two major ribosomal RNA bands is automatically determined and displayed with the RNA quantitation data on the electropherogram. An additional data evaluation tool is available for data comparison. The Agilent 2100 bioanalyzer contains high voltage power supplies, each of which is connected to a platinum electrode, which allows the instrument to perform multiple and precisely controlled separations. For the RNA applications, the instrument uses fluorescence detection, monitoring the fluorescence between 670 nm and 700 nm.

Chip preparation

All chips were prepared according to the chip preparation protocol provided with the RNA 6000 LabChip kit. The kit includes 25 chips, syringe, spin filters and the following reagents: sample buffer, gel matrix, and dye concentrate. In addition, the custom-made RNA 6000 ladder for use with the instrument/assay was purchased from Ambion, Inc. The gel-dye mix was prepared by mixing 400 µl of the gel matrix with 4 µl of the dye concentrate and filtering through a spin filter. The chip was filled with the gel-dye mixture and 5 µl of the sample buffer was then added to each sample well. 12 samples (1 µl each) in the concentration range from 10 to 500 ng/ µl were loaded into the sample wells of the chip. Finally, 1 µl of the RNA ladder was loaded into the assigned ladder well. The chip was then vortexed, and run on the Agilent 2100 bioanalyzer.

Reagents

Total mouse RNA was obtained from Ambion, Inc., USA and diluted in DEPC-treated water (Research Genetics Inc., USA). Total RNA (*E. coli*) was purchased from Roche Diagnostics Corp., USA. The RNA 6000 LabChip kit was obtained from Agilent Technologies GmbH, Germany. The custom-made RNA 6000 ladder for use with the RNA 6000 LabChip kit was purchased from Ambion, Inc.

Results and discussion

The RNA 6000 LabChip kit was used with the Agilent 2100 bioanalyzer to analyze various eukaryotic or prokaryotic total RNA samples. The software automatically calculates the ratio of the peak areas of the ribosomal bands, 18S/28S for eukaryotic and 16S/23S for prokaryotic samples. This information, in addition to the separation analysis, can be used as a parameter to estimate the quality of the preparation. An ideal total RNA preparation would result in a ratio of 2. Variability in this ratio may indicate partial degradation of the sample by ribonuclease contamination during the purification procedure. In the case of complete sample degradation these bands would disappear.

The analysis of twelve mouse total RNA samples from liver, spleen and heart tissues are shown in figure 1. The twelve samples were sequentially separated on the chip through a single separation channel and the resulting electropherograms are shown alongside the gel-like image. The chip run is completed within approximately 25 minutes. The data for each RNA sample can be accessed in real-time after each separation. Significant advantages over manual gel analysis are the data analysis and comparison functions, which are embedded in the software. No additional time consuming steps, such as scanning or densitometric analysis, are required.
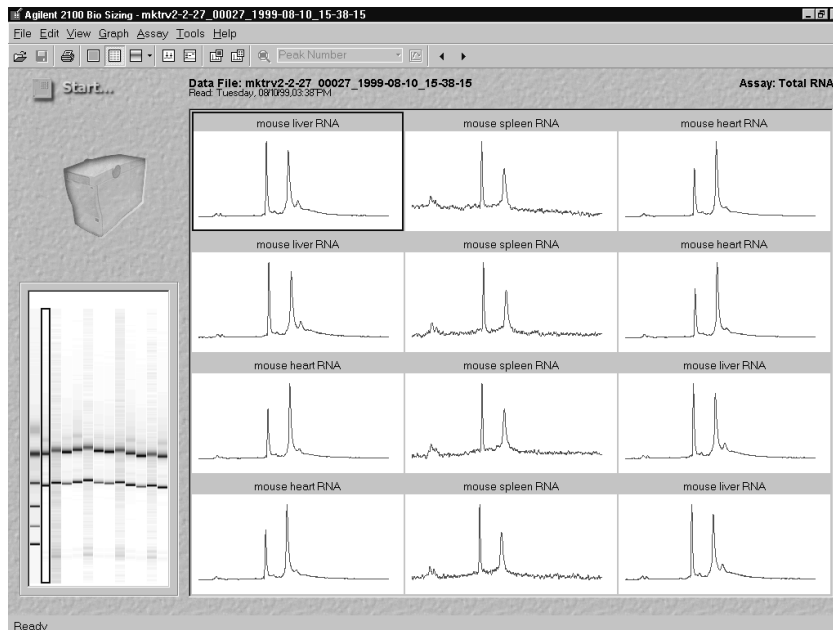

Figure 1
Agilent 2100 biosizing software. Data are displayed as electropherograms (samples 1-12) as well as a gel-like image. Various mouse total RNA samples (liver, spleen and heart) were separated from 12 different sample wells. The RNA 6000 ladder (first lane) is used as a reference.

RNA 6000 ladder standard

The RNA 6000 ladder standard is run on every chip from a specified ladder well and is used as a reference for data analysis. The RNA 6000 ladder contains six RNA fragments ranging in size from 0.2 to 6 kb at a total concentration of 150 ng/ µl (figure 2). The software automatically compares the unknown samples (1–12) to the ladder fragments to determine the concentration of the unknown samples and to identify the ribosomal RNA peaks. The ladder also serves as a built-in quality control measurement of system performance under standard conditions.

Quantitation accuracy of the RNA assay

The accuracy of the RNA assay was tested by running three different total RNA samples on 36 chips with n=36 for each sample. The total RNA samples tested were from liver, heart and spleen mouse tissues at concentrations ranging from approximately 10 to 500 ng/ µl as determined by UV absorption. Figure 3 shows the gel-like image and the electropherogram of one of the representative mouse heart total RNA samples analyzed. The table summarizes the quantitative data obtained for three total RNA samples analyzed on a chip in comparison to the concentrations measured by UV absorbance. Total RNA samples from different origins and concentrations can be separated on one chip and give good quantitation accuracy—the data was in the range of ± 35 % of the UV measured values. The total RNA separation with the Agilent 2100 bioanalyzer resulted in the identification of the 18S and 28S peaks for the eukaryotic total
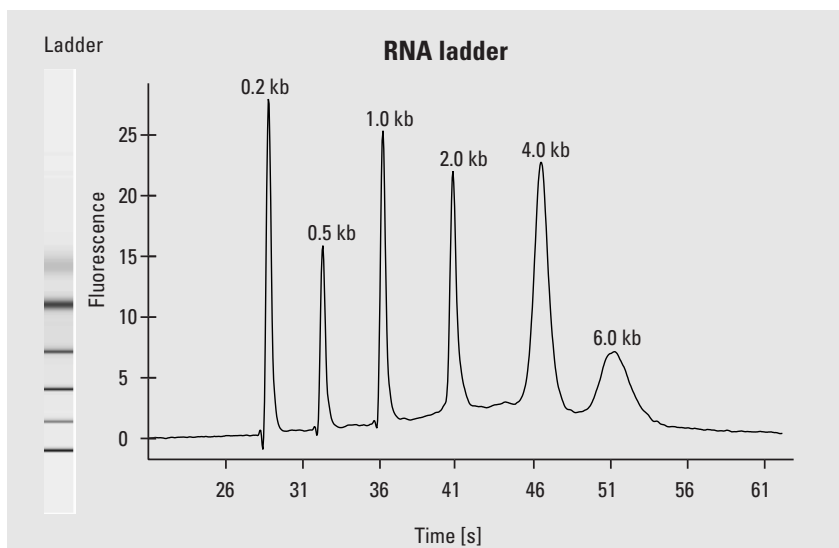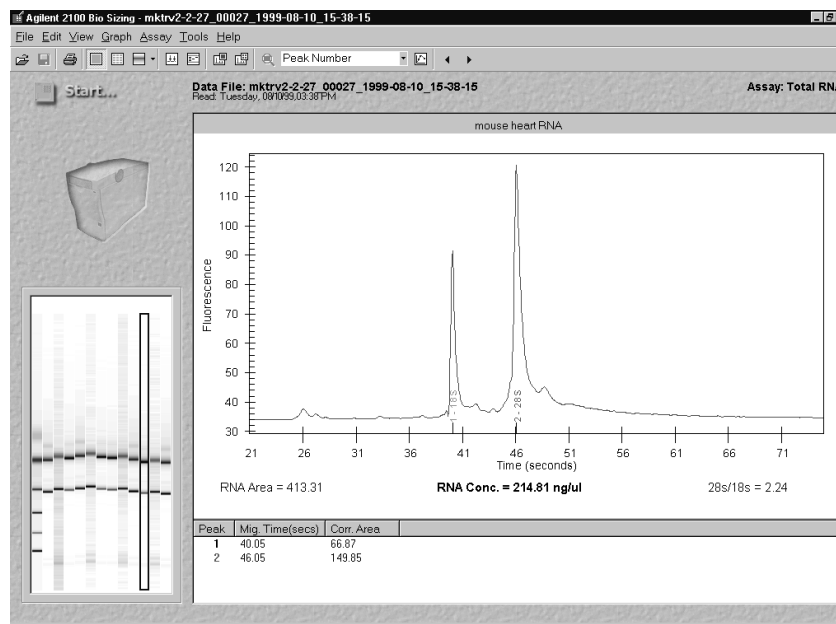

Figure 2
Electrophoretic separation of the RNA 6000 ladder using the RNA 6000 LabChip kit.

Total RNA samples	UV concentration (ng/µl)	Chip concentration (ng/µl)
mouse liver	77	70
mouse spleen	8	7
mouse heart	202	187

Figure 3
Representative example for the analysis of mouse heart total RNA. Mouse total RNA samples from different tissues and concentrations were separated with the Agilent 2100 bioanalyzer using the RNA 6000 LabChip kit (n=36) and compared to their actual concentration determined by UV measurement.

RNA and the separation pattern of these ribosomal peaks was reproducible within a chip and between chips. The expected effect of migration time variation due to the different sample origins was minimal when total RNA samples from different species, tissues, and concentrations were separated on the same chip (figure 3). The overlay of electropherograms from five chips with the data evaluation tool (figure 4-A) demonstrates only minimal variation between different chips. This data evaluation tool is especially valuable to compare the expression profile of distinct samples from different chips (figure 4-B).

Chip versus denaturing gel analysis of total RNA biopsy samples

Purified total RNA from different biopsy samples (1–8) were separated using the RNA 6000 LabChip kit (figure 5-A) and compared to the analysis of the same samples on a denaturing 2 % agarose gel with formaldehyde and stained with ethidium bromide (figure 5-B). The total RNA separation obtained under native conditions using the Agilent 2100 bioanalyzer gave comparable results with better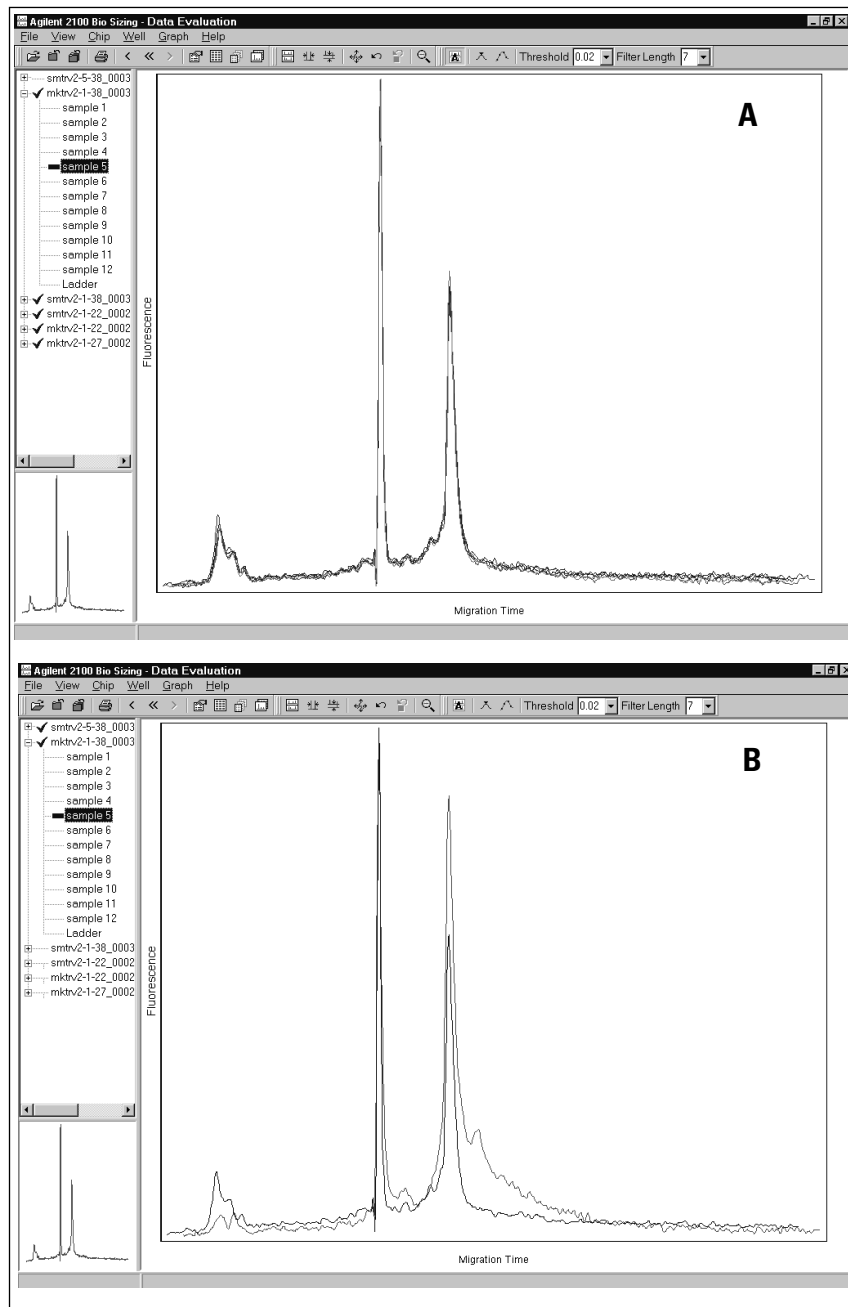 resolution compared to the agarose gel. The secondary structure has only a minor effect on the migration times under these conditions. Therefore, RNA separations under denaturing conditions are not required on the chip, avoiding exposure to hazardou sobtained under native conditions using the Agilent 2100 bioanalyzer gave comparable results with better resolution compared to the agarose gel. The secondary struc-

Figure 4
Comparison of electropherograms with the data evaluation tool. The electropherogram of mouse spleen total RNA (50 ng/µl) was compared from five different chips (A) and compared to 50 ng/µl mouse liver total RNA (B).

ture has only a minor effect on the migration times under these conditions. Therefore, RNA separations under denaturing conditions are not required on the chip, avoiding exposure to hazardous materials such as formaldehyde. An additional advantage of the chip-based total RNA analysis is the low sample volume requirement. 20 µl of total RNA (500 ng) were separated on the agarose gel in comparison to 1 µl (20–130 ng) of RNA sample which was separated on the chip. Because of the low sensitivity of ethidium bromide, a relatively high concentration of RNA had to be loaded onto the agarose gel in this experiment. In contrast, the combination of the intercalating dye used in the assay and the fluorescent detection used by the system enables detection of small concentration differences between samples that may not be detected using traditional gel electrophoresis. Additionally, the system provides a more precise quantitation of the RNA compared to the rough estimate obtained from gel electrophoresis.
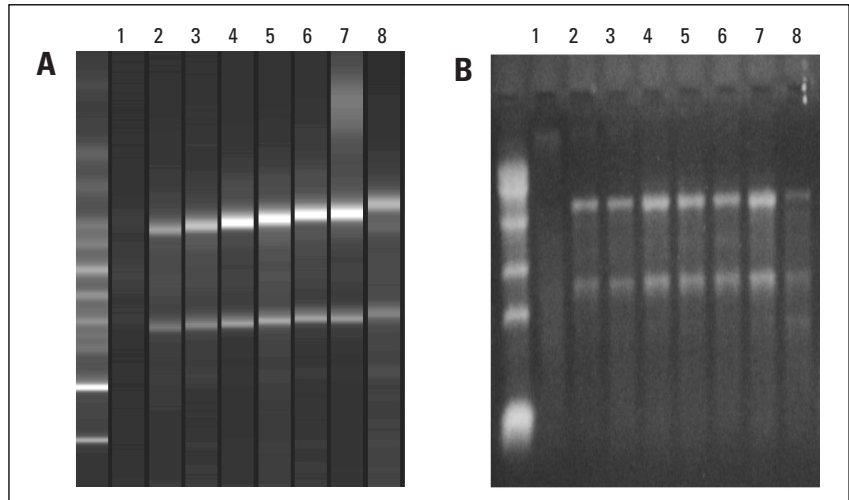

Figure 5
Comparison between total RNA separation from biopsy samples (1-8) on a 2 % agarose gel with formaldehyde and stained with ethidium bromide (B, reproduced with the permission of Chiron Corp.) with the separation obtained under native conditions using the Agilent 2100 bioanalyzer and the RNA 6000 LabChip kit (A).
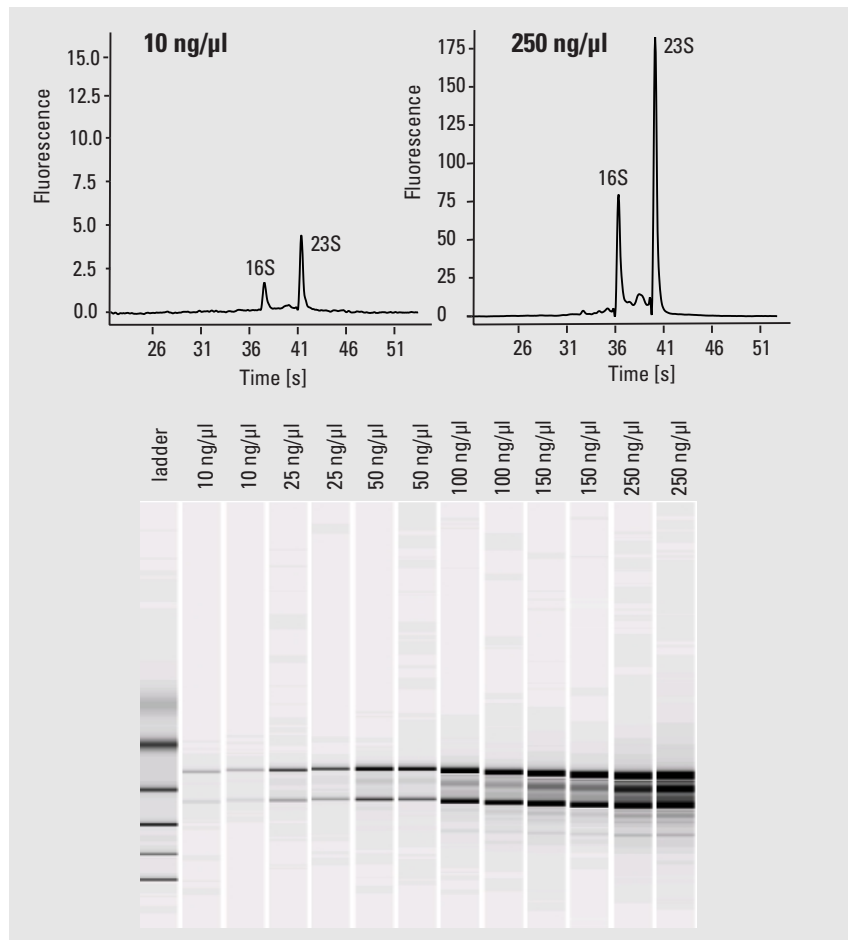

Figure 6
Representative example for the separation of different concentrations (10 to 250 ng/µl) of bacterial total RNA (*E. coli*) with the Agilent 2100 bioanalyzer.

Linear range of quantitation for bacterial RNA samples

Twelve dilutions of *E. coli* total RNA samples were analyzed to test the linear range and the detection limit of this assay for bacterial samples (figure 6). Total RNA can be detected over a broad range of 5 to 500 ng/µl and quantitated from 25 to 500 ng/µl. The Agilent 2100 biosizing software automatically ratios the 16S and 23S ribosomal bands of prokaryotic total RNA samples.

Conclusion

The LabChip-based total RNA analysis integrates the quantitation of RNA samples with a quality assessment in one quick and simple assay. The use of the Lab-on-a-Chip technology allows the rapid analysis of multiple samples through a single separation channel. Data precision is comparable or superior to agarose gel electrophoresis, whereas analysis time is greatly reduced. Automation of separation and data analysis makes the system versatile and results in less consumption of sample compared to conventional methods. In addition to total RNA analysis with the RNA 6000 LabChip kit, the instrument platform can also be used for other nucleic acid analyses. Initial kits are available for sizing of restriction fragment digests (DNA 12000 LabChip kit) and for sizing and quantitation of PCR fragments (DNA 7500 LabChip kit).

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Meike Kuschel is an application chemist based at Agilent Technologies, Waldbronn, Germany.

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