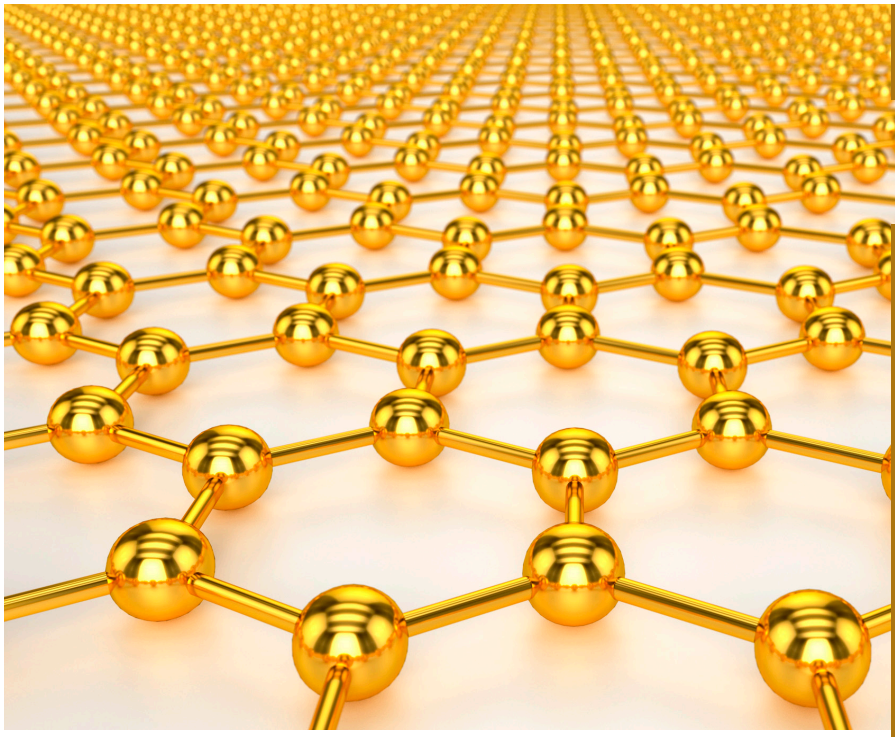

APPLICATION NOTE

ICP - Mass Spectrometry

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Accurate Sizing and Precise Counting of 10 nm Gold Nanoparticles using the Enhanced Sensitivity of the NexION 2000 ICP-MS

Introduction

The rapid development and implementation of nanomaterials in various consumer products has created the need to rapidly and accurately characterize nanoparticles (NPs) of various sizes and compositions. A number of techniques are available for the measurement of metal and metal-containing NPs, but all suffer from limitations[1]. The development of Single Particle ICP-MS (SP-ICP-MS) has overcome many of these limitations, and it has been well-documented as a technique to rapidly measure, count, and characterize metallic and metal-containing nanoparticles and/or nanomaterials[2-8]. According to the European Commission (EC) Recommendation (2011/696/EU), "nanomaterials" are defined as any "natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm"[9]. Traditionally, one of the challenges of nanoparticle (NP) analysis by SP-ICP-MS is the ability to accurately characterize particles that are smaller than 20 nm[10].

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In order to push below this 20 nm limit, a SP-ICP-MS instrument would require a combination of high sensitivity, fast data acquisition rates (< 75 µs dwell times), and low background. In addition, it is crucial to have an appropriate data processing software that is capable of automated threshold detection and real-time background subtraction[11]. In this work, we demonstrate that PerkinElmer's NexION® 2000 ICP-MS, with its unique RF generator and ion optics, coupled with the Syngistix™ Nano Application Software Module, can be used to accurately measure and characterize NP sizes of 10 nm and smaller, both alone and in a mixture of NPs of various sizes.

Experimental

Samples and Sample Preparation

The gold nanoparticle standards used for this work were NIST 8011 (10 nm), 8012 (30 nm), and 8013 (60 nm) (NIST, Rockville, Maryland, USA). All samples and standards were diluted using a diluent solution containing 2% isopropyl alcohol (IPA Sigma-Aldrich®, St. Louis, Missouri, USA) and 0.01% phosphate buffered saline (PBS, EMD Chemicals, Billerica, Massachusetts, USA) in deionized water; the IPA and PBS were used to maintain the stability of the gold (Au) nanoparticles.

Instrumentation

All analyses were performed using a NexION 2000 ICP-MS (PerkinElmer Inc., Shelton, Connecticut, USA), with the conditions listed in Table 1. The instrument was optimized using the automated SmartTune™ procedure focusing only on nebulizer gas flow rate and targeting maximum Au sensitivity. The Au signal was measured in Reaction mode using pure ammonia as a gas, benefitting from the unique collisional focusing effect, one of the many advantages of the NexION 2000's patented Universal Cell Technology™ (UCT). Transport efficiency was measured using NIST 8013 and found to be 9.82%. All data were acquired and processed using the Syngisitix Nano Application Module (PerkinElmer Inc.), the easiest and most intelligent SP-ICP-MS software processing package available on the market, featuring automated threshold detection, real-time background subtraction, and multiple point calibrations from both ionic and particulate standards.

Table 1. NexION 2000 ICP-MS Operating Conditions.

Parameter	Value
Nebulizer	Concentric (MEINHARD® plus Glass, Type C)
Spray Chamber	Glass Cyclonic at 2 °C
Injector	2.5 mm id
Nebulizer Flow	1.14 L/min
Plasma Gas Flow	18 L/min
RF Power	1600 W
Dwell Time	50 µs
Analysis Time	10-120 s
Reaction Gas	Ammonia at 0.45 mL/min

Results and Discussion

In order to obtain maximum sensitivity for Au, the analysis was performed in Reaction mode. This mode allows the user to take advantage of the collisional focusing effect offered by the NexION 2000's Universal Cell Technology (UCT). Gold sensitivity increased by a factor of two compared to non-Reaction mode. Experimentally, it was found that maximum Au sensitivity was obtained with an ammonia flow of 0.45 mL/min.

Once the system is optimized and the transport efficiency determined, the next step for nanoparticle analysis is to perform both dissolved and particle calibrations. Figures 1 and 2 show both of these calibration curves. The dissolved curve (Figure 1) was made with 0.080, 0.125, and 0.275 µg/L Au standards, and yields a correlation coefficient of 0.99955, demonstrating the linear response at low concentrations. The particle calibration curve was made with NIST 8011, 8012, and 8013 using Transmission Electron Microscope (TEM) reported values of 8.9, 27.6, and 56 nm, respectively. Figure 2A shows the particle calibration at full scale, but only two points are visible: the 30 and 60 nm NP standards. Zooming in on the lower part of the curve (Figure 2B), shows that the 10 nm Au NPs are visible and in-line with the 30 nm NPs. Further expanding the calibration curve around the 10 nm NP (Figure 2C) clearly shows that it is visible above the origin. Overall, these results demonstrate the ability to clearly detect 10 nm Au NPs and attest to the linearity of the particle calibration curve from 10 to 60 nm NPs.

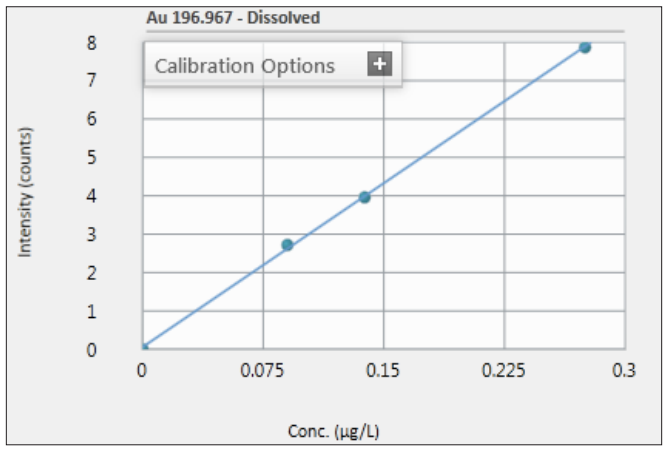

Figure 1. Dissolved calibration plot for 0.080, 0.125, and 0.275 µg/L Au.

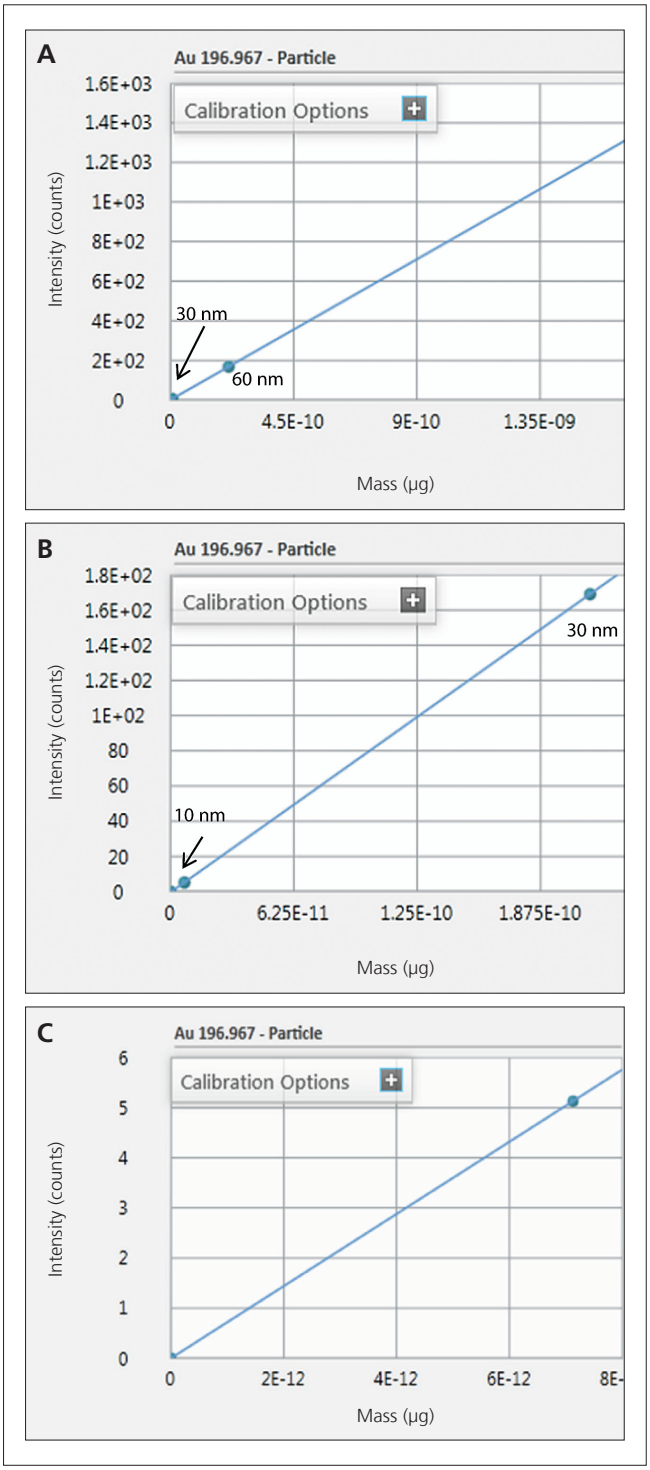

Figure 2. Particle calibration for 10, 30, and 60 nm Au NPs (A); Zoomed-in particle calibration showing 10 and 30 nm Au NPs (B); Zoomed-in particle calibration showing 10 nm Au NP and origin (C).

Figure 3 shows the real-time plots of both a blank (A) and 10 nm Au NPs (B). In both plots, the x-scale is zoomed in to show that individual peaks can be seen. Comparing both plots clearly shows that 10 nm NPs can easily be detected above the blank. The algorithm did not detect any NPs in the blank, demonstrating the ability to differentiate small particles from the background.

Figure 4 shows the particle size distribution determined for the NIST 8011 10 nm Au NPs, with a Gaussian fit to the distribution. The certificate lists an actual particle size of 8.9 nm, as determined by Transmission Electron Microscopy and 8.5 nm by Atomic Force Microscopy. Both of these values agree with the SP-ICP-MS values of 8 and 8.92 nm for most frequent size and median size, respectively. These data demonstrate the ability to measure particle sizes below 10 nm accurately, with a method quantitation size (MQS) of 4 nm. The MQS is calculated based on the conversion of one count above the threshold to size. Figure 3 also shows that particle sizes are binned in 1 nm increments, meaning that size differences as small as 1 nm can be accurately determined. This high selectivity can only be achieved by using peak area integration rather than peak height. This, in turn, highlights the need for fast data acquisition speeds in order to have enough points per peak to properly integrate particles below 20 nm. Based on the peak widths, a minimum data acquisition speed of 50 µsec/point is necessary to achieve proper sampling points.

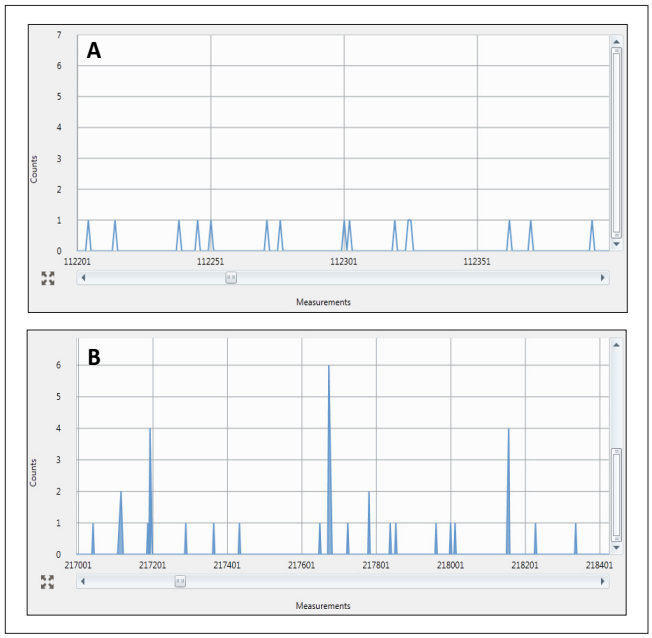

Figure 3. Real-time trace for a blank (A) and 10 nm Au NPs (B), with the x-axis zoomed so that individual acquisition points can be seen.

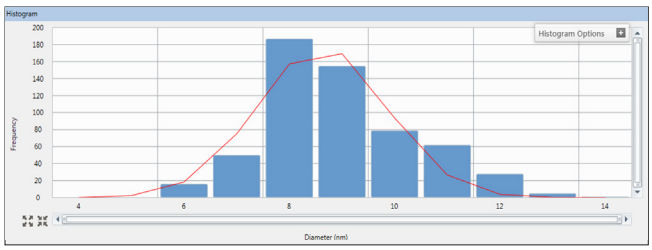

Figure 4. Particle size distribution for NIST 8011 10 nm Au NPs, fitted with a Gaussian distribution.

To further validate the methodology, measurements were performed for five different acquisition times, ranging from 10-120 seconds (three measurements made at each time). The results in Table 2 demonstrate that acquisition times as low as 10 sec can be used to generate accurate data: the mean size, mean intensity, and average particle concentration are all equivalent, with RSDs of 3% or less. The average particle size determined is 8.9 nm, which agrees with the TEM value of 8.9 nm.

To further validate the results and test the counting precision, sequential dilutions of the NIST 8011 10 nm were performed and analyzed. The results in Table 3 demonstrate that high-quality data can be achieved even at relatively low particle concentrations (2500 part/mL). Accurate sizing was achieved regardless of the dilution factor used. These data further validate the methodology and provide concrete evidence on the ability of the NexION 2000 ICP-MS to accurately measure and precisely count particles smaller than 10 nm.

Table 2. Counting Precision of NIST 8011 10 nm Au NPs.

Analysis Time (Sec)	Mean Size (µm)	Mean Intensity (Counts)	Avg. Particle Conc. (Part/mL)	% RSD (n=3)
10	9.02	6.75	21307	2.91
20	9.04	6.80	21502	4.09
30	8.94	6.69	21229	2.92
60	8.78	6.26	20319	2.45
120	8.83	6.46	20467	3.76
Average	8.92	6.59	20695	
% RSD	0.90	3.25	2.33	

Table 3. Measured Size as a Function of Particle Concentration for NIST 8011 10 nm Au NPs.

Initial Particle Concentration = 20,000 part/mL						
Dilution Factor	Expected Conc. (Part/mL)	Measured Conc. (Part/mL)	% RSD (n=3)	% Deviation (Absolute)	Measured Size (nm)	% RSD
1	20,000	20,681	2.51	3.29	8.8	0.90
2	10,000	9,759	3.50	2.55	8.9	0.77
4	5,000	5,015	2.55	0.29	8.8	0.56
8	2,500	2,431	3.74	2.84	8.9	0.14

Ionic Versus Particle Calibration

A major hurdle against expanding the applicability of SP-ICP-MS is the lack of particle standards necessary to perform many metallic and metal oxides nanoparticle studies. In order to overcome such limitation, the NexION 2000's Syngistix Nano Application Module includes a patented routine whereby particle size can be determined using either dissolved or particle calibration curves[11]. To demonstrate the accuracy of this technique, the calibration curves in Figure 1 were used to determine the particle sizes of the three NIST reference materials, with the results appearing in Table 4. It can be clearly seen from the data (result of 3 analyses of each sample) that the dissolved calibration produced equally accurate results for the three sizes when compared to particle calibrations. The size distribution histograms for the NIST 8011 10 nm Au NPs from both the ionic and particle calibrations are shown in Figure 5, validating the ability to characterize NPs using only ionic standards.

Table 4. Particle Size Results Determined with Particle and Dissolved Calibrations.

Sample	Certificate Value (by TEM; nm)	Particle Calibrations (nm)	Dissolved Calibrations (nm)
NIST 8011	8.9 ± 0.1	8.8 ± 0.03	8.8 ± 0.03
NIST 8012	27.6 ± 0.1	27.8 ± 0.06	27.9 ± 0.03
NIST 8013	56 ± 0.51	56.1 ± 0.37	56.2 ± 0.30

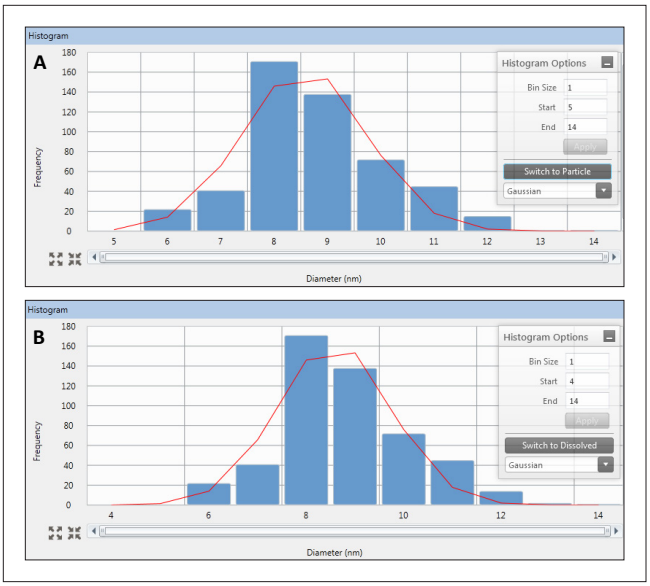

Figure 5. Size distribution histograms for NIST 8011 10 nm Au NPs using an ionic/dissolved calibration (A) and particle calibration (B).

Analysis of a Particle Mixture

With the ability to accurately measure sub-10 nm Au NPs established, the ability to measure these NPs in a mixture of different-sized NPs was investigated. The mixture composition is shown in Table 5, with the total NP concentration limited to 100,000 part/mL to avoid coincidence. Figure 6 shows the size distribution histogram for this mixture, along with the integration region for each peak. The experimentally obtained data are displayed in Table 6 and show that the results are both accurate and precise. These results demonstrate the ability to measure a mixture of different size NPs and determine the size and the concentration (part/mL) of each component. The resolution between the peaks allows for accurate size determinations within the mixture, an important capability since nanoparticle mixtures rarely contain single-size particles. The differing peak widths shown in Figure 6 highlight the importance of being able to specify the integration window for each peak in order to obtain accurate results from a nanoparticle mixture. This feature is required in any nanoparticle handling software, otherwise samples will have to be re-run multiple times.

Table 5. Composition of Au NP Mixture.

Component	Concentration (Part/mL)
10 nm Au NPs	20,000
30 nm Au NPs	50,000
60 nm Au NPs	30,000

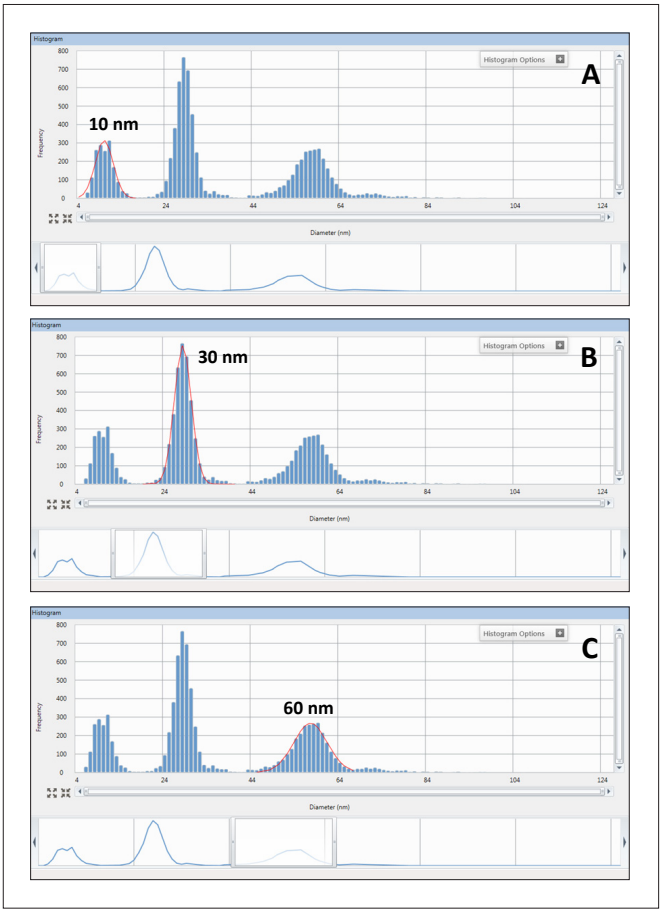

Figure 6. Size distribution histograms for a mixture of 10, 30, and 60 nm Au NPs. This average particle size determined from integrating each peak is shown on the histogram. Integration of the: 10 nm peak (A); 30 nm peak (B); 60 nm peak (C).

Table 6. Analysis of a Mixture of Different Nanoparticle Sizes.

	NIST 8011 - 10 nm			NIST 8012 – 30 nm			NIST 8013 – 60 nm		
Replicate	Most Frequent Size (nm)	Mean Size (nm)	Part. Conc. (Part/mL)	Most Frequent Size (nm)	Mean Size (nm)	Part. Conc. (Part/mL)	Most Frequent Size (nm)	Mean Size (nm)	Part. Conc. (Part/mL)
1	9	8.9	20,058	28	28.3	49,411	57	56.6	31,295
2	9	8.9	20,622	28	28.3	49,391	57	56.7	31,007
3	9	8.7	21,327	28	28.2	49,763	57	57.0	31,585
Average	9	8.8	20,669	28	28.3	49,522	57	56.8	31,296
% RSD	0.0	1.3	3.1	0.0	0.2	0.4	0.0	0.4	0.9

Conclusion

This work has demonstrated the ability of PerkinElmer's NexION 2000 ICP-MS coupled with the Syngistix Nano Application Software Module to accurately measure NPs smaller than 10 nm using either dissolved/ionic or particle calibrations. The method quantitation size was found to be 4 nm, and the size distribution histograms showed 1 nm resolution, allowing for accurate measurements in 1 nm size increments. When a mixture of different particle sizes are analyzed, each size distribution is easily seen and can be individually integrated without having to analyze the sample multiple times to gain information for each component.

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Consumables Used

Component	Description	Part Number
Gold (Au) Nanoparticles	60 nm, 2.60E+10 part/mL, 25 mL	N8142303
Aqueous Gold (Au) Standard	1000 mg/L, 125 mL	N9303759
Sample Uptake Tubing	Orange/green (0.38 mm id) flared PVC, pack of 12	N0777042
Drain Tubing	Gray/gray (1.30 mm id), Santoprene, pack of 12	N0777445
Sample Tubes	Box of 500	B0193233 (15 mL) B0193234 (50 mL)

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