Laboratory supplies

Factors to consider in accuracy and precision of Nalgene Dropper Bottles

Introduction

Using dropper bottles to dispense liquids

Dropper bottles are an alternative to pipetting devices that allow for consistent, accurate, repeated reagent dispensing in a dropwise fashion. They feature a dropper control tip that snaps securely into place to provide a leakproof seal. Dropper bottles made of opaque or translucent low-density polyethylene (LDPE) have excellent **chemical resistance** and are suitable for most biotechnology, diagnostic, and pharmaceutical applications. They are used for point-of-care applications such as instilment devices for eye and ear drops, medication dosing, contact lens solutions, and cosmetics, and for dispensing sanitizing solutions such as alcohol or hydrogen peroxide. They are also used as parts of self- or bedside-diagnostic kits (e.g., SARS-CoV-2 antigen test kits, pregnancy tests), and to dispense adhesives for dental crowns, auto-polymerizing resins, craft glue, paint, or even light machine oil.

Instillation of conventional aqueous solutions generally results in the delivery of drops that are accurate and precise to the denoted volume of the dropper bottles. However, in the pharmaceutical industry dropper bottles are often used to instill solutions that contain viscolysers (water-soluble, synthetic or semi-synthetic polymers, polysaccharides, cellular esters, and microbial polysaccharides) to increase the contact time of eye drops with the ocular surface and to increase the bioavailability and efficacy of the drug [1,2]. These agents can drastically vary the density and viscosity of a solution, resulting in low accuracy or precision of the droplets delivered by the dropper bottle.

Likewise, in biotechnology applications, dropper bottles are utilized to deliver lateral flow chromatographic buffers that often contain surfactants such as the ionic detergent sodium dodecyl sulfate (SDS) or Tween™ 20, Triton™ X-100, or Brij™ 35 nonionic agents.

When the dispensing reagent deviates from the physicochemical properties of water and aqueous solutions, there is a possibility that the droplet volume is neither accurate nor precise [3]. In cases where a customer is using a solution that contains surfactants or detergents, the droplet's surface tension is reduced, which can lead to dispensing droplets that are significantly lower in volume than the denoted delivery volume [4]. Since the solution's viscosity and/or surface tension may impact the accuracy and/or precision of the volume of solution dispensed by the dropper bottle, users should be aware of potential inaccuracies and imprecision of droplets that may arise as a result of the liquid's physicochemical properties.

Some manufacturers denote the volume that the dropper bottles deliver. However, this information is commonly based on the dispensing of water and may not accurately represent the volume dispensed for other solutions. Therefore, we investigated the effects of the physical properties (solution density, surface tension, and viscosity) of fluids upon the accuracy and precision of dispensing under standard laboratory conditions at room temperature (RT) and 4°C. This technical note investigates the effect of physicochemical properties on the accuracy and precision of LDPE dropper bottles.

Experimental design

Evaluating differences in accuracy and precision among dropper bottles from various suppliers

To evaluate the importance of careful selection when considering a dropper bottle among suppliers, similar LDPE dropper bottles from five different manufacturers were tested to determine their accuracy and precision. The percentage (%) accuracy was defined as the percentage of the proximity of a droplet measurement to the true (denoted) tip volume. The precision was the degree to which repeated droplet measurements under the same conditions showed the same droplet volume (reproducibility).

First, double-distilled H2O (ddH2O) droplet weights were measured and volumes were calculated. ddH_2O was used as the nonviscous, non–surface-active reference solution, as it is commonly used when testing the performance of devices that measure the volume of liquid, due to its well-characterized physicochemical properties (Table 1). All dropper bottles were filled to their maximum volume capacity to eliminate any variability from the residual volume of liquid in the dropper bottle [5]. Dropper bottles were equilibrated at room temperature (RT, 24–25°C) for 4 hr. The drop volume of the tested solution was calculated by its weight, which was determined using a calibrated analytical precision weight scale (Mettler Toledo) with 0.001 g readability under standard conditions. The weight was converted to volume using the formula $d = m/V$, where d is the density of the tested solution (g/mL), m is the mass (g), and V is the volume (mL).

Other studies show that the dispensing angle and the residual volume of liquid in the dropper bottles are two factors that affect the accuracy and precision of dropper bottles. There is a significant effect from the dispensing angle that is highly influenced by the user's manipulation at the moment of use.

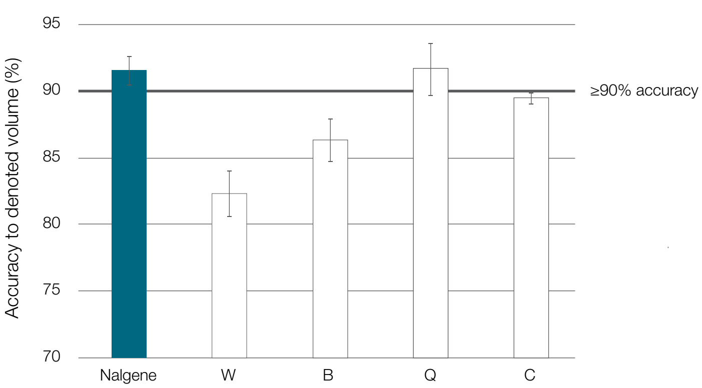
Previous research shows that a dispensing angle less than 48° from horizontal resulted in a significant increase in drop weights that was attributed to the drop formation on the wetted external surface of the tip orifice. Dispensing angles greater than 48° did not affect drop weight [5]. In addition, others also determined that the effect of residual volume in the dropper bottle was not found to be significant [6]. Based on these literature data, and to exclude any variability stemming from the dispensing angle and the residual volume, all measurements in this study were performed with dropper bottles filled to their maximum capacity and held at 90° from horizontal.

Measurement of 10 different droplets from 3 different dropper bottles was performed by 2 different technicians (total $N = 60$ measurements per each supplier's dropper bottle for each solution and temperature) and compared to the denoted droplet volume. Accuracy and precision data were analyzed with a Student's t-test. A p-value of 0.05 or less was considered significant. Standard errors were calculated based on standard deviations of the ten droplet volumes and are reflected as error bars in all graphs of the study. To confirm the comparisons that were $p < 0.05$ with the Student's t-test, one-way ANOVA with Tukey's honestly significant difference (HSD) analysis was also performed for all pairwise comparisons of the mean values to separate groups. A value of $q < 0.05$ was considered significant.

Results

Factors that affect accuracy and precision of dropper bottles

Results with water (Figure 1) show that the Thermo Scientific™ Nalgene™ Dropper Bottle performed with high accuracy (91.6%) and high precision (CV 4.01%) compared to supplier Q's bottle, which similarly shows high accuracy (91.7%) but lower precision (CV 7.38%). Dropper bottles from suppliers W, B, and C displayed lower accuracy of <90%. Also, bottles from suppliers W and B had greater CV values.



Dropper bottle	Accuracy (%)	CV (%)	p-value
Nalgene	91.6	4.01	1.0000
W	82.3	7.19	0.1328
B	86.3	6.41	0.3293
Q	91.7	7.38	0.9874
C	89.5	1.61	0.4996

Figure 1. Accuracy and precision of dropper bottles using ddH_2O. Dropper bottles were filled with ddH_2O to their maximum capacity. The droplet volume accuracy test was carried out at a vertical (90°) angle, with a dispensing rate of 1 drop per 10 seconds. The y-axis shows the percentage of the proximity of a droplet measurement to the true (denoted) tip volume. The values for mean accuracy are given in the table; the error bars represent the standard error (precision of delivered droplets). The variability of the droplet volumes is expressed by the coefficient of variation (CV). The line indicates 90% accuracy, the threshold for acceptable values. A Student's t-test was performed to determine if the differences in the performance of dropper bottles were statistically significant ($p < 0.05$).

Second, measurements of droplet volumes were performed with a 1% (v/v) glycerol aqueous solution to mimic liquids found in biomedical applications, such as eye drops and contact lens solutions. Since eye drops and contact lens solutions can be stored at RT or in the refrigerator, the dropper bottles were tested at both 24–25°C and 4°C (liquid temperatures). The physical properties of these solutions at 24–25°C and 4°C are noted in Table 1. Figure 2 shows droplet accuracy and precision comparisons at the two test temperatures for Nalgene Dropper Bottles and dropper bottles from suppliers W, B, Q, and C. Measurements were taken after the tested solution and dropper bottles were equilibrated for 4 hr at the corresponding temperatures. The accuracy of both Nalgene and supplier Q dropper bottles with the glycerol solution was consistently >90% at both RT and 4°C (Figure 2). The dropper bottles of suppliers W, B, and C performed with <90% accuracy at RT but >90% at 4°C. Statistical evaluation with the Student's t-test showed that the dropper bottles of suppliers W, B, and C at RT were significantly less accurate than Nalgene Dropper Bottles and supplier Q ($p < 0.05$). Among the top performers, Nalgene Dropper Bottles had higher precision compared to supplier Q's dropper bottles (CV 2.01% vs. 4.18%) at 4°C.

Effect of surface tension

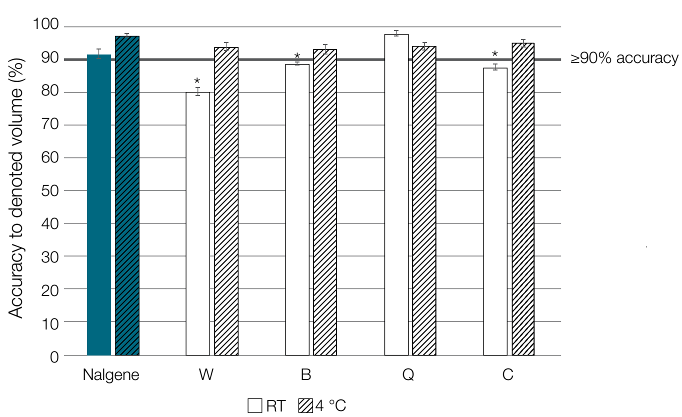
A phosphate solution containing Tween 20 detergent (1X DPBS/T, 8 mM sodium phosphate, 2 mM potassium phosphate, 0.14 M NaCl, 10 mM KCl, 1% Tween 20 detergent, pH 7.4) was chosen to mimic ophthalmic solutions that contain viscolysers. These agents are added to increase the contact time of medications used to treat glaucoma, antibiotics to treat bacterial eye infections, and antihistamines and mast cell stabilizers to relieve eye itching and redness [7]. Also, a similar solution is being utilized as a chase buffer in lateral-flow immune-chromatographic rapid antigen/antibody diagnostic tests for HIV, syphilis, and SARS-CoV-2. The 1X DPBS/T has a high surface activity that lowers droplet surface tension compared to ddH_2O and 1% glycerol solution.

DPBS/T has a density of 1.016 g/mL, calculated surface tension of 69.5 mN/m, and viscosity of 0.8882 cPoise at RT (Table 1). The reference ddH_2O has a density of 1.000 g/mL, surface tension of 72 mN/m, and viscosity of 1.0019 cPoise at RT. The DPBS/T results in Figure 3 showed that dropper bottles from all manufacturers performed with <90% accuracy and CV <10%. As the DPBS/T solution had a lower surface tension (69.5 mN/m) than water (72 mN/m), we observed that the accuracy decreased following the same trend as the decrease in surface tension. The addition of a surfactant such as Tween 20 detergent lowered the surface tension and resulted in smaller droplets that were released more readily than the ddH_2O solution from the dropper bottle nozzle.

Table 1. Solutions tested with each dropper bottle, and their physicochemical properties.

Solution	Concentration	Room temp. 24–25°C			4°C		
		Density (g/mL)	Calculated* viscosity (cPoise)	Calculated* surface tension (mN/m)	Density (g/mL)	Calculated* viscosity (cPoise)	Calculated* surface tension (mN/m)
ddH_2O	100%	1.0000	1.0019	72.0	0.9756	1.3080	74.1
Glycerol	1%	1.0033	1.0119	72.8	0.9801	1.3211	74.9
DPBS/T	1X	1.0160	0.8882	69.5	0.9902	1.1596	71.5
HEMA	25%	1.0300	1.525	50.5	1.0121	1.9909	52.0

* Viscosity and surface tension values are approximate and are based on values reported in the literature [8-11].



*p-value <0.05, significant compared to Nalgene Dropper Bottle at 4°C.

Dropper bottle	RT			4°C		
	Accuracy (%)	CV (%)	p-value	Accuracy (%)	CV (%)	p-value
Nalgene	91.6	5.76	0.2305	97.2	2.01	1.0000
W	80.2	5.19	0.0063	93.7	4.49	0.3468
B	88.7	1.53	0.0072	93.0	5.51	0.3357
Q	97.8	2.63	0.8115	93.8	4.18	0.3312
C	87.6	3.20	0.0162	94.8	4.04	0.4630

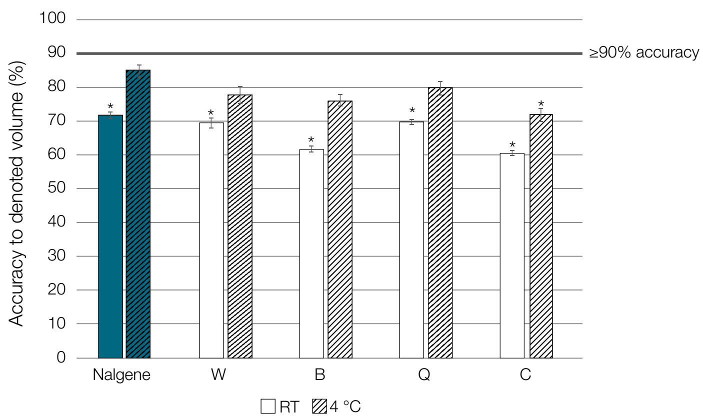
Figure 2. Accuracy and precision of dropper bottles using 1% glycerol solution. The test was performed at room temperature (RT, 24–25°C) and 4°C. A Student's t-test indicated that the droplet volumes at RT for bottles of suppliers W, B, and C were statistically different from those of the Nalgene Dropper Bottle. At 4°C, there were no significant differences in droplet volumes among suppliers.

Effect of viscosity

A 25% (v/v) 2-hydroxyethyl methacrylate (HEMA) solution was chosen as an example of the highest viscosity and lowest surface tension; this agent is often used in dental adhesion applications and composite resins. This solution had a density of 1.030 g/mL, calculated surface tension of 50.5 mN/m, and viscosity of 1.525 cPoise. The results with 25% HEMA (Figure 4) showed also that dropper bottles from all manufacturers performed with <90% accuracy and CV of <6%. The inaccuracy with 25% HEMA was greater than with the DPBS/T solution. As 25% HEMA has higher viscosity than water, we observed that the percent accuracy decreased as the viscosity increased.

Effect of temperature

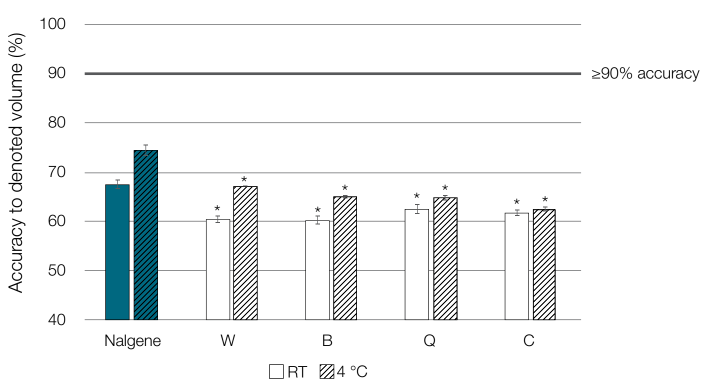
It is noteworthy that there was a difference in the accuracy of the dropper bottles between RT and 4°C. With the glycerol solution, the dropper bottles of suppliers W, B, and C performed with <90% accuracy at RT but >90% accuracy at 4°C. This was also observed with both DPBS/T and 25% HEMA—all five suppliers' bottles were more accurate at 4°C than at RT. As temperature decreased from 25°C to 4°C, the surface tension and viscosity of the solutions increased, resulting in the formation of more accurate droplets.



*p-value <0.05, significant compared to Nalgene Dropper Bottle at 4°C.

Dropper bottle	RT			4°C		
	Accuracy (%)	CV (%)	p-value	Accuracy (%)	CV (%)	p-value
Nalgene	71.7	2.89	0.0094	84.9	3.99	1.0000
W	69.7	3.60	0.0070	77.9	5.75	0.1528
B	61.9	2.51	0.0010	76.2	3.98	0.0546
Q	70.3	1.69	0.0045	80.1	4.30	0.2341
C	60.7	2.07	0.0007	72.1	4.78	0.0199

Figure 3. Accuracy and precision of dropper bottles using 1X DPBS/T solution. The phosphate buffer solution is often used as a chase buffer in point-of-care rapid antigen/antibody lateral flow immune-chromatographic diagnostic tests (HIV, syphilis, SARS-CoV-2). The 1X DPBS/T is also similar in composition with some eye dropper bottle solutions that contain viscolysers to extend the contact of the active ingredient of eye drops with the ocular surface. Analysis shows that solutions that contain surface-active reagents such as Tween 20 detergent result in inaccurate drop volumes. Storage of these solutions in a refrigerator can restore to some extent the accuracy of droplet volumes to the true (denoted) tip volume.



*p-value <0.05, significant compared to Nalgene Dropper Bottle at 4°C.

Dropper bottle	RT			4°C		
	Accuracy (%)	CV (%)	p-value	Accuracy (%)	CV (%)	p-value
Nalgene	67.6	4.87	0.1134	74.7	5.04	1.0000
W	60.5	3.63	0.0100	67.3	0.14	0.0485
B	60.4	4.51	0.0120	65.0	1.56	0.0243
Q	62.6	4.57	0.0223	64.9	2.70	0.0283
C	61.8	2.23	0.0295	62.4	3.63	0.0165

Figure 4. Accuracy and precision of dropper bottles using 25% HEMA. This solution had the highest calculated viscosity (1.525 cPoise at RT and 1.9909 cPoise at 4°C) and the lowest calculated surface tension (50.5 mN/m at RT and 52 mN/m at 4°C) of all the solutions tested. These two factors negatively affected the accuracy of dropper bottle volumes. Data show that users should consider the physical properties of their solutions and determine the impact they may have on the accuracy and precision of their application.

Although dropper bottles from all suppliers performed with <90% accuracy with DPBS/T and 25% HEMA, Nalgene Dropper Bottles performed consistently better than those of suppliers W, B, Q, and C. This demonstrated that not all dropper bottles are the same, and therefore customers should consider this, especially when using dropper bottles with reagent solutions other than water. Furthermore, in applications where it matters, such as in dosing of pharmaceuticals, it is recommended that dropper bottles be kept near 4°C to ensure the best possible accuracy in droplet dosage.

Conclusion

These results illustrate the performance differences among dropper bottles from different manufacturers and the importance of these differences when choosing dropper bottles for applications where accuracy and precision are important, such as dosing of biopharmaceuticals. The higher the accuracy and the lower the CV, the safer it is to use these dropper bottles in dosage-sensitive applications such as ocular instillation of antihistamines, antibiotics, and glaucoma medication.

There are many different options when it comes to purchasing dropper bottles. The present study shows that not all dropper bottles are the same when it comes to applications that require high accuracy and precision. To avoid the introduction of systemic errors in applications where the accuracy and precision of the volume dispensed is important, users should be mindful of their selection of dropper bottles for their specific application.

Another key consideration is the physicochemical properties of solutions and to determine the impact they may have on the accuracy and precision of dropper bottles. For applications requiring the use of solutions containing surfactants, or fluids that have high viscosity, users should understand that these properties will reduce the accuracy of the volume being dispensed and potentially affect the application. Another relevant factor is the temperature at which the dropper bottle is used and stored and the effects this may have on the volume dispensed.

In some cases, knowing the effects these factors have on the accuracy of the volume dispensed, users can make adjustments, such as changing the concentration of the active ingredient, making changes to the formulation of the solution to be dispensed, or changing the temperature at which the dropper bottle is to be used and stored, to help improve the accuracy of the volume. For example, dispensing reagents such as chase buffer solutions that contain Tween 20 detergent at 4°C can help to improve the accuracy of the dispensed volume. For solutions that contain a combination of reagents with higher viscosity and/or surfactants, it is recommended that the users conduct their own testing and adjust the number of drops to achieve the correct dosing if accurate volumes are critical in their specific application.

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Ordering information

Product	Quantity	Cat. No.
Nalgene LDPE 4 mL Natural Dropper Bottle	Case of 2,000	312750-9125
Nalgene LDPE 4 mL White Dropper Bottle	Case of 2,000	312751-9125
Nalgene LDPE 8 mL Natural Dropper Bottle	Case of 2,000	312750-9025
Nalgene LDPE 8 mL White Dropper Bottle	Case of 2,000	312751-9025
Nalgene LDPE 15 mL Natural Dropper Bottle	Case of 2,000	312750-9050
Nalgene LDPE 15 mL White Dropper Bottle	Case of 2,000	312751-9050
Nalgene LDPE Dispensing Tips for Dropper Bottles 40 µL	Case of 2,000	312759-0001
Nalgene LDPE Dispensing Tips for Dropper Bottles 50 µL	Case of 2,000	312758-0001
Nalgene LDPE 4 mL White Dropper Bottle with 40 µL Tip and Closure	Case of 25	2751-9125
Nalgene LDPE 8 mL White Dropper Bottle with 40 µL Tip and Closure	Case of 25	2751-9025
Nalgene LDPE 15 mL White Dropper Bottle with 40 µL Tip and Closure	Case of 25	2751-9050
Nalgene LDPE 15 mL White Dropper Bottle with 40 µL Tip and Assorted Closures	Case of 25	2753-9050
Nalgene LDPE Bottle with Dropper Assembly 60 mL	Case of 48	2416-0060
Nalgene LDPE Bottle with Dropper Assembly 60 mL	Pack of 12	2416-0060PK
Nalgene LDPE Bottle with Dropper Assembly 125 mL	Case of 48	2416-0125
Nalgene LDPE Bottle with Dropper Assembly 125 mL	Pack of 6	2416-0125PK
Nalgene 4 mL Natural Dropper Bottle with 40 µL Tip and White Closure	Case of 25	2750-9125
Nalgene 4 mL Natural Dropper Bottle with 40 µL Tip and Assorted Closures	Case of 25	2752-9125
Nalgene 8 mL Natural Dropper Bottle with 40 µL Tip and White Closure	Case of 25	2750-9025
Nalgene 8 mL Natural Dropper Bottle with 40 µL Tip and Assorted Closures	Case of 25	2752-9025
Nalgene 15 mL Natural Dropper Bottle with 40 µL Tip and White Closure	Case of 25	2750-9050
Nalgene 15 mL Natural Dropper Bottle with 40 µL Tip and Assorted Closures	Case of 25	2752-9050

References

1. Meseguer G, Buri P, Plazonnet B et al. (1996) Gamma scintigraphic comparison of eyedrops containing pilocarpine in healthy volunteers. *J Ocul Pharmacol Ther* 12(4):481–8.
2. Van Santvliet L, Ludwig A (1999) Influence of the physico-chemical properties of ophthalmic viscolysers on the weight of drops dispensed from a flexible dropper bottle. *Eur J Pharm Sci* 7(4):339–45.
3. Brown D, Ford JL, Nunn AJ et al. (2004) An assessment of dose-uniformity of samples delivered from paediatric oral droppers. *J Clin Pharm Ther* 29(6):521–9.
4. Sijs R, Kooij S, Bonn D (2019) How surfactants influence the drop size in sprays. arXiv:1907.09723
5. Sklubalová Z, Zatloukal Z (2006) Study of eye drops dispensing and dose variability by using plastic dropper tips. *Drug Dev Ind Pharm* 32(2):197–205.
6. Moore DB, Beck J, Kryscio RJ (2017) An objective assessment of the variability in number of drops per bottle of glaucoma medication. *BMC Ophthalmol* 17(1):78.
7. Kumar S, Karki R, Meena M, et al. (2011) Reduction in drop size of ophthalmic topical drop preparations and the impact of treatment. *J Adv Pharm Technol Res* 2(3):192–4.
8. Segur JB, Oberstar HE (1951) Viscosity of glycerol and its aqueous solutions. *Ind Eng Chem* 2117–2120.
9. Montheard JP, Chatzopoulos M, Chappard D (1992) 2-hydroxyethyl methacrylate (HEMA): Chemical properties and applications in biomedical fields. *J Macromol Sci Part C* 32(1):1–34.
10. Takamura K, Fischer H, Morrow NR (2012) Physical properties of aqueous glycerol solutions. *J Petrol Sci Eng* 98–99:50–60.
11. Tavana H, Kuo C-H, Lee QY et al. (2010) Dynamics of liquid plugs of buffer and surfactant solutions in a micro-engineered pulmonary airway model. *Langmuir* 26(5):3744–52.

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