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ADVANCED INSTRUMENTS, INC.

Performance Characteristics of a New Single-Sample Freezing Point Depression Osmometer

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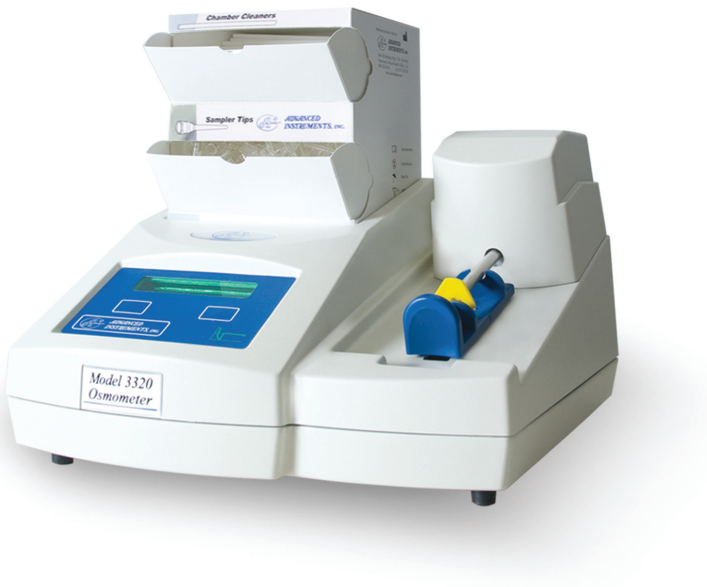
ABSTRACT

The Advanced® Model 3320 is an automated, single-sample freezing point depression (FPD) micro-osmometer that determines the total solute concentration (osmolality) of biological fluids, such as serum or urine. Osmolality measurements are commonly used by clinicians to assist in diagnosing and monitoring certain fluid and electrolyte imbalances in patients (i.e., hyponatremia, polyuria). FPD osmometers have been used in clinical chemistry laboratories for more than 40 years and provide a direct measurement of osmolality.

This study was conducted to evaluate linearity and imprecision performance parameters of the new Advanced Model 3320 Micro-Osmometer using 20 µL samples of commercially available calibrators, reference solutions, controls, and normal human serum. Five replicates of 5 different osmolality levels ranging from 100 to 2,000 mOsm/kg H_2O were tested to assess linearity. Short and long-term replication experiments were conducted to evaluate imprecision. For the short-term study, 5 replicates of the following solutions were tested over a period of 20 days: 290 mOsm/kg H_2O reference solution; 850 mOsm/kg H_2O standard; urine matrix 300 mOsm/kg H_2O; urine matrix 800 mOsm/kg H_2O; serum matrix 240 mOsm/kg H_2O; serum matrix 320 mOsm/kg H_2O; and normal human serum. The long-term study lasted 547 days and was conducted by testing 10 replicates of the 290 mOsm/kg H_2O reference solution at various time intervals, for a total of 37 days of testing. The calculated slope and y-intercept of the linearity test were 0.99 and 1.5, respectively.



The following short-term total standard deviation (SD) results were obtained: 1.3SD, 290 mOsm/kg H_2O reference solution; 2.8SD, 850 mOsm/kg H_2O standard; 1.6SD, urine matrix 300 mOsm/kg H_2O; 3.0SD, urine matrix 800 mOsm/kg H_2O; 1.5SD,

serum matrix 240 mOsm/kg H_2O; 1.5SD, serum matrix 320 mOsm/kg H_2O; and 2.2SD, normal human serum. In the long-term study, the mean value and total SD for the 290 mOsm/kg H_2O reference solution were 290.6 and 1.5, respectively.

The linearity results of the osmometer indicate a strong linear response with a slope close to 1 and an intercept close to zero. The total standard deviations for all samples tested in both the short and long-term studies were within the manufacturer's imprecision claims of ± 2 mOsm/kg H_2O (1SD) between 0 and 400 mOsm/kg H_2O, and ± 0.5% (1SD) between 400 and 2,000 mOsm/kg H_2O. The linearity and imprecision results generated on the Advanced Model 3320 Micro-Osmometer correlate well to its specifications and demonstrate excellent instrument performance.

INTRODUCTION

One of the major changes in the CLIA Final Rule is that laboratories must now perform validation studies on all non-waived methods implemented after April 24, 2003. According to the Code of Federal Regulations, Title 21, Volume 8, 21 CFR Section 862.2730, "An osmometer for clinical use is a device intended to measure the osmotic pressure of body fluids. Osmometers determine osmotic pressure by methods such as the measurement of the freezing point. Measurements obtained by this device are used in the diagnosis and treatment of body fluid disorders."

Osmolality is considered a moderate complexity analyte, and both moderate and high complexity methods are now combined under the new term "non-waived". CLIA requirements for method validation of clinical osmometers include the verification of performance specifications, as outlined in section 493.1253 (b) (1), prior to reporting patient test results.

In this study, we assessed linearity and imprecision performance characteristics on the Advanced Model 3320 Micro-Osmometer following CLSI protocol guidelines.

MATERIAL AND METHODS

Linearity and short and long-term replication experiments to evaluate imprecision were conducted by testing 20 µL samples on the Advanced Model 3320 Micro-Osmometer (Advanced Instruments, Inc.) following manufacturer's instructions. Five replicates of 5 different osmolality levels ranging from 100 to 2,000 mOsm/kg H_2O (Osmolality Linearity Set, 3LA028, Advanced Instruments, Inc.) were tested to assess linearity.

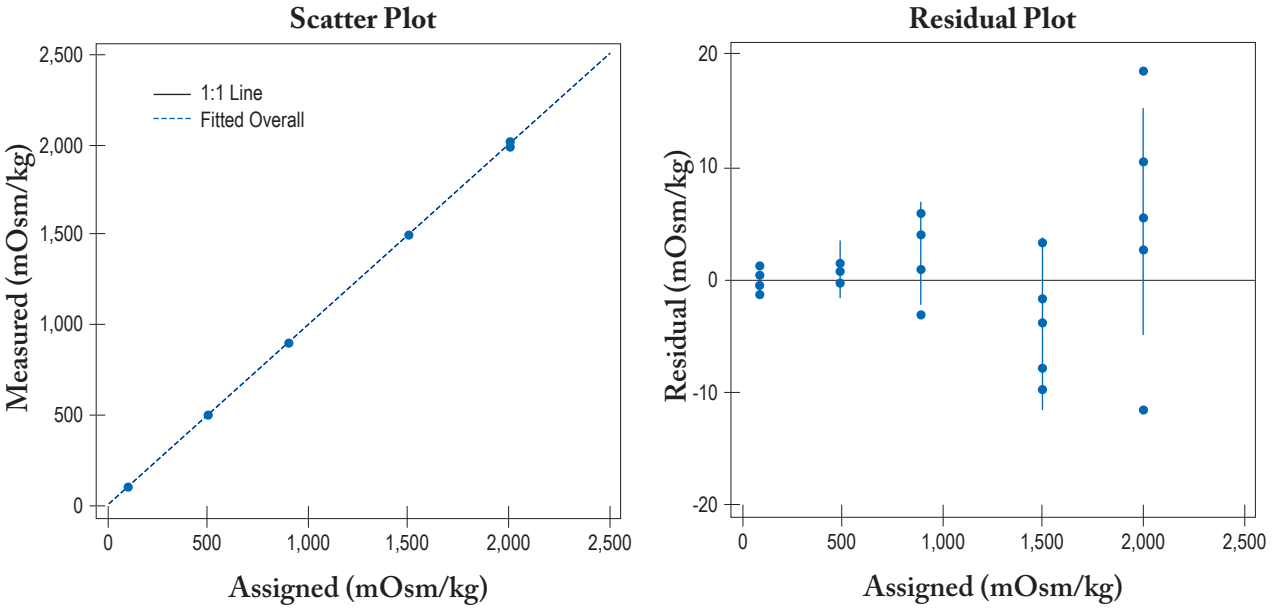
For the short-term study, 5 replicates of the following commercially available solutions from Advanced Instruments, Inc., were tested over a period of 20 days: 290 mOsm/kg H_2O reference solution (3MA029); 850 mOsm/kg H_2O standard (3MA085); urine matrix 300 mOsm/kg H_2O (3LA085); urine matrix 800 mOsm/kg H_2O (3LA085); serum matrix 240 mOsm/kg H_2O (3MA028); serum matrix 320

mOsm/kg H_2O (3MA028); and normal human serum. The long-term replication experiment lasted 547 days and was conducted by testing 10 replicates of the 290 mOsm/kg H_2O reference solution (3MA029) at various time intervals, for a total of 37 days of testing.

Linearity (slope and intercept) and short-term precision parameters (mean and total standard deviation) were evaluated statistically using EP Evaluator Release 7.0.0.251 (David G. Rhoads Associates, Inc.). The total SD is a composite of within-run, between-run, and between-day SD. Long-term precision data was evaluated using Minitab® (Minitab, Inc.). A total of 369 data points were included and one excluded due to a "sample did not freeze" error. The long-term precision data was also evaluated using ControlChart!Pro® Plus version 7.13.02 (ChemSW, Inc.) by plotting the average values of 10 data points for each day, using limits based on the mean ± 3 standard deviations. One outlier was removed from this data set.

RESULTS

Fig. 1. Linearity summary using 5-level osmolality set, showing measured versus assigned values, slope and intercept.



Linearity Summary

	N	Slope	Intercept	Error
Overall	5	0.999	1.5	0.3%

LINEAR within Allowable Systematic Error of 0.5%

Statistical Analysis and Experimental Results

	Assigned	Est	Mean	Resid	Linear?	Measured Concentrations				
a	100	101.5	101.2	-0.3	Pass	101	100	103	102	100
b	500	501.3	502.2	0.9	Pass	502	503	501	503	502
c	900	901.0	903.4	2.4	Pass	907	902	898	905	905
d	1500	1500.7	1496.8	-3.9	Pass	1499	1491	1497	1493	1504
e	2000	2000.4	2000.8	5.2	Pass	2003	1989	2011	2006	2019

Table. 1. Short-term total standard deviation results, n=20

Sample	Mean (SD)
290 mOsm/kg H_2O reference solution	290.7 (1.3)
850 mOsm/kg H_2O standard	851.2 (2.8)
urine matrix 300 mOsm/kg H_2O	300.4 (1.6)
urine matrix 800 mOsm/kg H_2O	803.9 (3.0)
serum matrix 240 mOsm/kg H_2O	240.4 (1.5)
serum matrix 320 mOsm/kg H_2O	319.0 (1.5)
normal human serum	286.9 (2.2)

Fig. 2. Long-term precision data descriptive statistics.

Variable: Result	
Anderson-Darling Normality Test	
A-Squared	7.867
P-Value	0.000
Mean	290.569
StDev	1.490
Variance	2.21872
Skewness	0.363742
Kurtosis	0.470413
N	369
Minimum	287.000
1st Quartile	290.000
Median	290.000
3rd Quartile	291.000
Maximum	296.000
95% Confidence Interval for Mu	
290.417	290.722
95% Confidence Interval for Sigma	
1.389	1.606
95% Confidence Interval for Median	
290.000	291.000

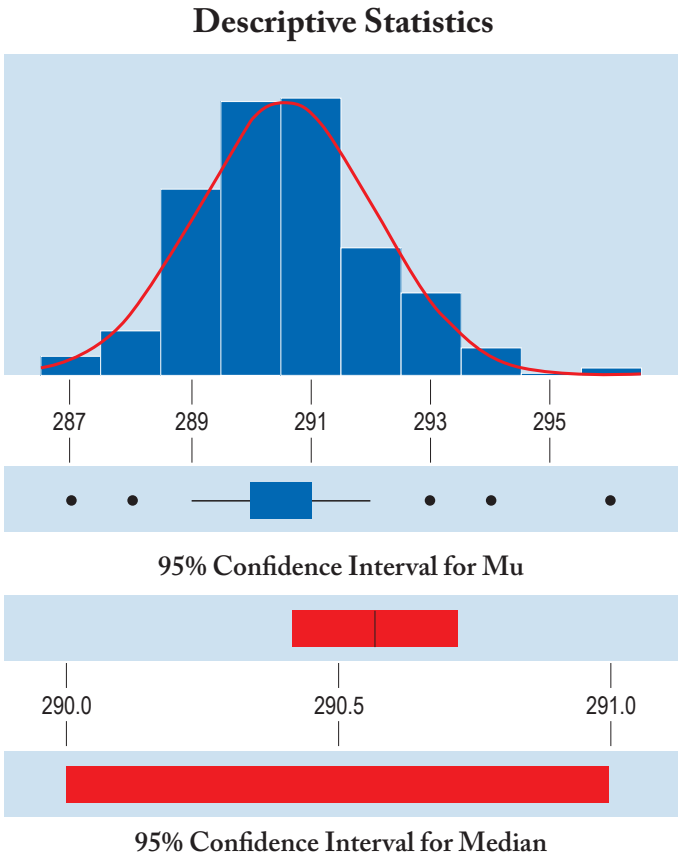
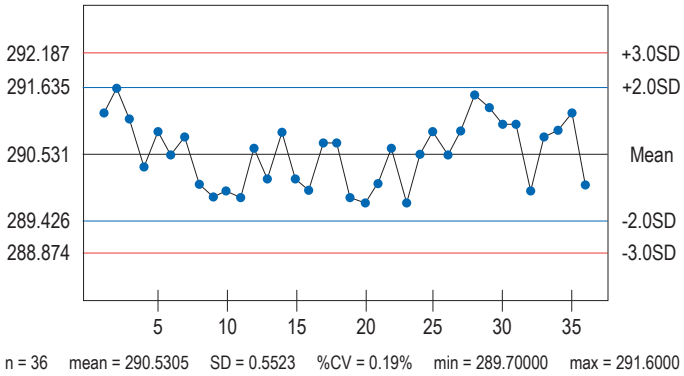


Fig. 3. Control chart depicting average values for each day of long-term testing using limits based upon the mean ± 3 standard deviations.



CONCLUSION

NIST-traceable reference salt solutions and CLSI-recommended matrix control materials formulated to mimic unknown specimens were used in this study. The linearity results of the osmometer indicate a strong linear response with a slope close to 1 and an intercept close to zero (Fig. 1). The total standard deviations for all samples tested in both the short (Table 1) and long-term (Fig. 2) studies were within the manufacturer's imprecision claims of ± 2 mOsm/kg H_2O (1SD) between 0 and 400 mOsm/kg H_2O, and ± 0.5% (1SD) between 400 and 2,000 mOsm/kg H_2O. The instrument was calibrated only once at the beginning of the long-term drift study, indicating that this instrument is extremely stable (Fig. 3). The linearity and imprecision results generated on the Advanced Model 3320 Micro-Osmometer correlate well to its specifications (Fig. 4) and demonstrate excellent instrument performance.

Fig. 4. Model 3320 Single-Sample Micro-Osmometer specifications.

Model 3320 Single-Sample Micro-Osmometer Specifications	
Sample Volume	20 µL
Test Time	60 seconds
Sample Capacity	Single Sample
Units	mOsm/kg H_2O
Resolution	1 mOsm/kg H_2O
Range	0 to 2000 mOsm/kg H_2O
Communications	DTE RS-232 serial port, parallel printer port, and optional barcode scanner
Performance at Reference Conditions[1]	
Linearity	Less than ± 1% from a straight line between 0 and 2000 mOsm/kg H_2O
Repeatability	± 2 mOsm/kg H_2O (1 S.D.) between 0 and 400 mOsm/kg H_2O; ± 0.5% (1 S.D.) between 400 and 2000 mOsm/kg H_2O
Drift	Less than 1 mOsm/kg H_2O per month
Performance at Operating Conditions	
Temperature Effects	Less than 1 mOsm/kg H_2O per 5°C (9°F) ambient temperature range
[1]Reference Conditions	20 to 25°C (68 to 77°F); 40 to 60% relative humidity; tolerances of reference or calibration solutions excluded

REFERENCES

1. CLSI. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition, CLSI document EP05-A2 (ISBN 1-56238-542-9). CLSI, 2004.

2. CLSI. Statistical Quality Control for Quantitative Measurement Procedures; Approved Guideline – Third Edition, CLSI document C24-A3 (ISBN 1-56238-613-1). CLSI, 2006.

3. Westgard J. Basic Method Validation, Second Edition, 2003.

4. Westgard J, Ehrmeyer, S, Darcy, T. CLIA Final Rules for Quality Systems, First Edition, 2004.

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