The MX3 Hydration Testing System

Summary of Findings:

- Salivary Osmolarity (SOSM) is a sensitive marker of change in hydration status after exercise.
- SOSM measurements from the MX3 Hydration Testing System show excellent concordance with laboratory osmometer SOSM measurements.
- Field testing of the MX3 Hydration Testing System confirms the ease of conducting MX3 SOSM measurements on large groups of athletes and demonstrates increasing SOSM values with increasing loss of body mass due to exercise induced dehydration.

The MX3 Hydration Testing System

Physical and mental performance is dependent upon maintaining appropriate hydration.
Water loss of 1-2% body weight compromises aerobic performance. Water loss of >2% body weight reduces muscular endurance and increases the risk of heat exhaustion and heat stroke[1].

While there is currently no scientific consensus on how to best assess and monitor hydration status in athletes, there are many tools that have been described for assessment of dehydration. The gold standard of hydration assessment in sports science and medical contexts is the estimation of total body water through isotope dilution and measurement of plasma osmolarity. However, these techniques are analytically complex, expensive and invasive, making them impractical for continuous monitoring of hydration status[2].

More commonly, a combination of self-reported thirst, urine specific gravity (USG) and nude percentage body mass loss (%BML) are used to assess hydration in athletes due to the accessibility of this information[3]. While %BML is recognised as an effective method of assessing acute changes in hydration status due to water loss, frequent measurement is impractical and urinary measures have been shown to be imprecise in an active dehydration setting[4,5]. Salivary osmolarity (SOSM) has been proposed as an alternative non-invasive measure for field monitoring of hydration, with multiple studies showing that changes in SOSM provide a more accurate measure of acute dehydration than urine testing when measured alongside %BML[6–8].

Despite this evidence, SOSM has been considered an impractical method for field testing of hydration status due to the cost, lack of portability and technical expertise associated with using laboratory-based osmometers[9]. Using the MX3 Hydration Testing System, SOSM can be measured in only a few seconds by tapping a disposable test strip against an individual's tongue or collected saliva sample **(Figure 1)**.

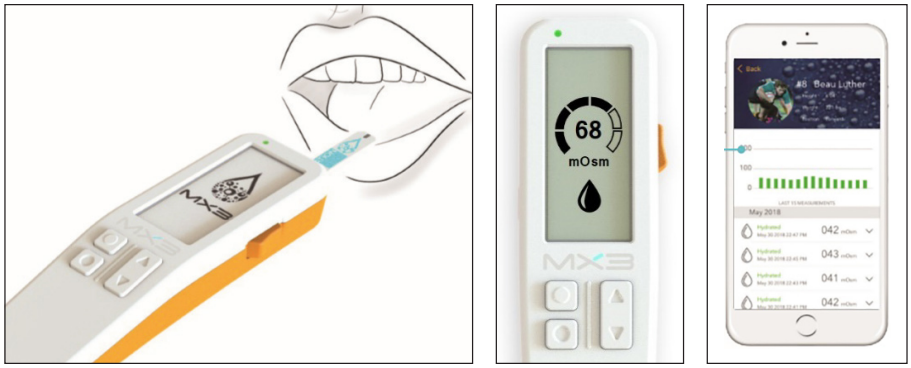


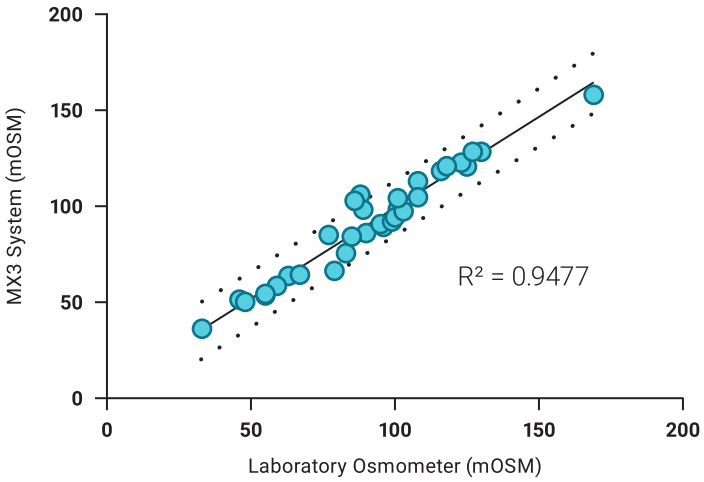
Figure 1

Taking a SOSM measurement with the MX3 Hydration Testing System

Accuracy of measurements taken with the MX3 Hydration Testing System

SOSM measurements made with the MX3 Hydration Testing System have an excellent correlation (R2= 0.95) with SOSM values determined by a laboratory certified osmometer **(Figure 2)**.

Figure 2 - Correlation of Osmolarity Readings

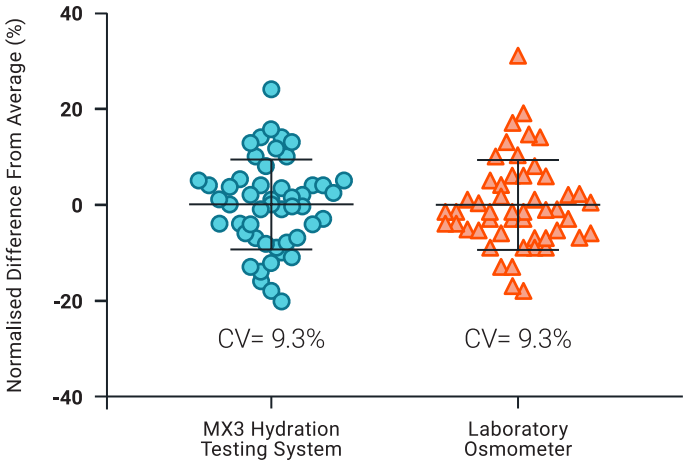


Correlation of average SOSM readings for 33 saliva samples using the MX3 hydration testing system and an Osmette III commercial osmometer. Dotted lines represent 95% prediction bands.

Precision of measurements taken with the MX3 Hydration Testing System

SOSM measurements made with the MX3 Hydration Testing System have an excellent precision. Replicate readings on 33 saliva samples resulted in an average coefficient of variation (CV) in osmolarity of 3.3% (± 2.1%). In practice, greater variability is observed when performing replicate measurements of saliva samples directly from the tongue due to biological variation in saliva production. When measuring stimulated saliva samples with a consistent collection method, some variation in SOSM (+/- 15%) is normal and is observed with both the MX3 system and a laboratory grade osmometer **(Figure 3)**.

Figure 3 - Stimulated Saliva Sampling Variation



Typical variation in SOSM readings as determined using the MX3 Hydration Testing System and a Laboratory Osmometer.

Applications of Salivary Osmolarity

Multiple studies have demonstrated a strong relationship between SOSM and changes in body mass **(Figure 4)**. Walsh *et al.*[6] **(Figure 4A)**, Smith *et al.*[7] **(Figure 4B)**, and Munoz *et al.*[8] **(Figure 4C)** showed that salivary osmolarity increased relative to increased BML during active exercise trials, with the most prominent effects observed at BML > 2%. Stookey *et al.*[10] **(Figure 4D)** demonstrated that maintained increase in water intake over multiple weeks could increase body water content, which was reflected in SOSM. These studies confirm the ability of SOSM to track both acute and chronic changes in hydration state.

Figure 4 - Applications of Salivary Osmolarity

*Figure 4A - Saliva Parameters as Potential Indices of Hydration Status during Acute Dehydration (Walsh, 2004)**

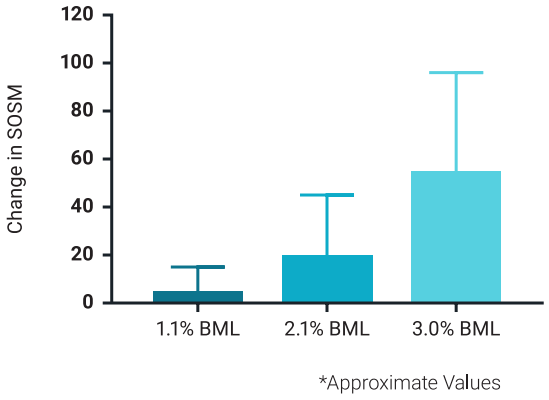


Figure 4B - Use of Salivary Osmolarity to Assess Dehydration (Smith, 2012)

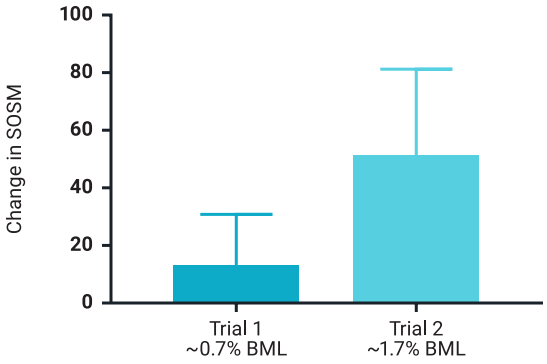


*Figure 4C - Assessment of hydration biomarkers including salivary osmolality during passive and active dehydration (Munoz, 2013)**

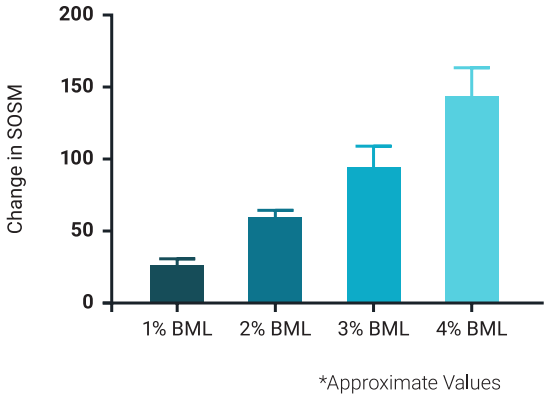
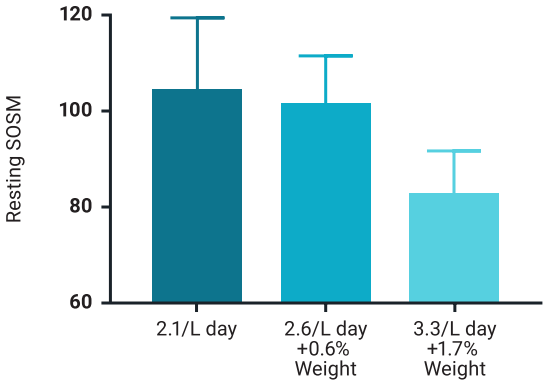


Figure 4D - Change in hydration indices associated with an increase in total water intake (Stookey, 2017)



Field testing of the MX3 Hydration Testing System

To confirm the relationship between SOSM and %BML, MX3 has conducted an extensive field test performing SOSM measurements using the MX3 system before and after exercise with a fluid restriction protocol, conducted alongside laboratory-grade SOSM testing using a commerical osmometer, nude body weight and USG measurements (Table 1). MX3 hydration measurements were taken on the pitch, in a gym or in the locker room—environments where urine or nude body mass measurements would not normally have been possible.

This largest study of its kind to date replicated previously observed relationships between SOSM and %BML, whereby increased SOSM values were highly correlated with increased %BML following SOSM mesaures made using both the MX3 System (R^2 = 0.303, p = 0.004) and a laboratory grade osmometer (R^2 = -0.370, p = 0.001). In the same study population, no relationship was observed between USG and %BML (R^2 = 0.069, p = 0.522). These results demonstrate that the MX3 Hydration Testing System can deliver laboratory-grade results in the field and characterise changes in hydration status due to exercise-induced dehydration significantly more effectively than measuring USG.

Table 1 - Field Testing of the MX3 Hydration Testing System

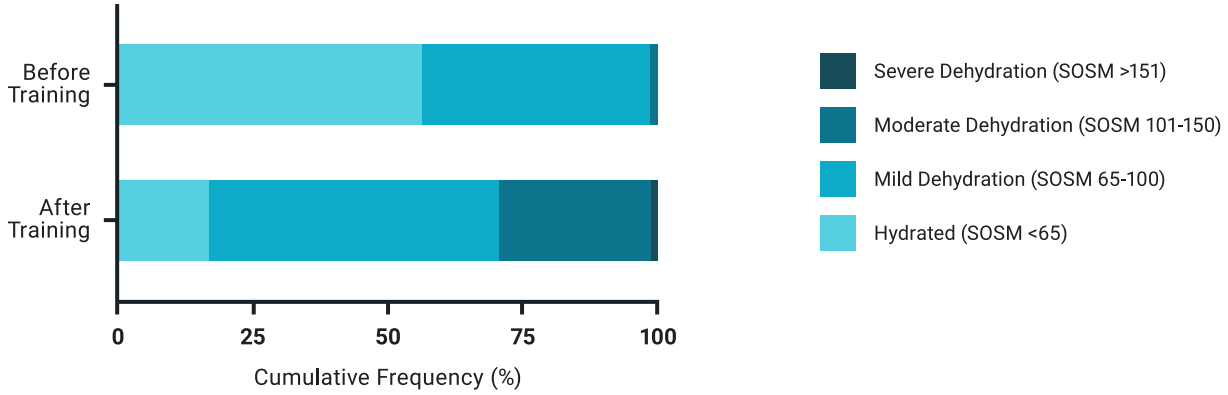
	N = 90	N = 84	N = 88
	SOSM Change (MX3 System)	**SOSM Change (Laboratory)**	**USG Change**
Correlation with %BML	0.303	0.370	0.069
Significance	0.004	0.001	0.522

SOSM change is significantly correlated with %BML when measured with either the portable MX3 system or a laboratory osmometer.

MX3 Hydration Testing at the Melbourne Marathon Training Club

MX3 measurements were offered to athletes before and after seven training sessions in the lead-up to the 2018 Melbourne Marathon. Over 250 hydration measurements were performed on more than 130 athletes. This study found that before exercise a large proportion of athletes were hydrated (56%) or only mildly dehydrated (42%) as determined by SOSM. In contrast, after exercise almost all athletes were mildly dehydrated (53%) or moderately dehydrated (28%), and a small subset were severely dehydrated (2%) **(Figure 5)**.

Figure 5 - Data collected across 7 training sessions of marathon training group (n = 272)

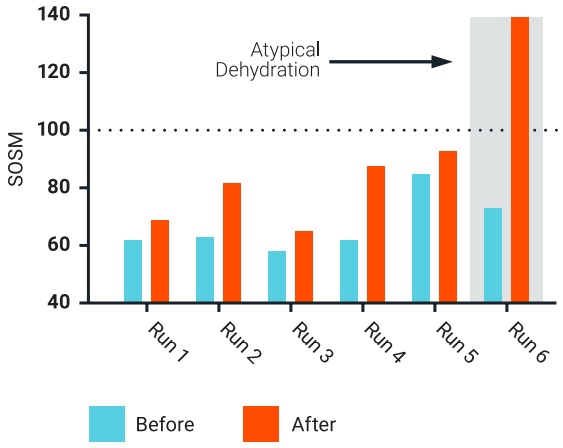


SOSM measurements demonstrate a clear shift towards a dehydrated state following training.

Paired hydration measurements taken from the same athlete across multiple runs demonstrated a potential to identify training sessions where this athlete became atypically dehydrated **(Figure 6A)**. Measurements from multiple athletes during the same training session demonstrated the potential of SOSM measurements to identify athletes who had become particularly dehydrated by a high intensity training run **(Figure 6B)**.

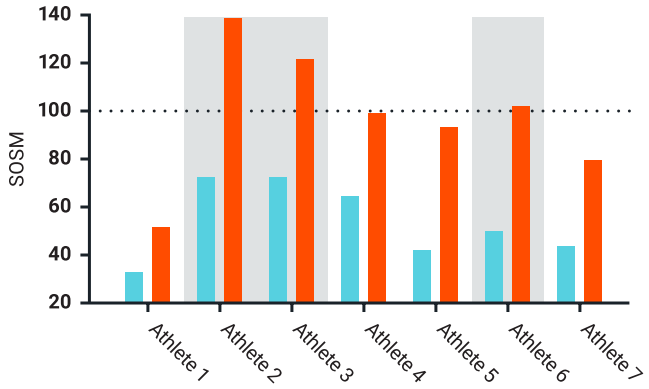
Figure 6 - Paired hydration measurements of athletes during marathon training sessions

Figure 6A - Example of measurements of a single athlete across multiple training runs



These measurements could identify sessions where the athlete became atypically dehydrated (SOSM > 100) and should be directed to focus on rehydration.

Figure 6B - Example of paired measurements of multiple athletes during a single training run

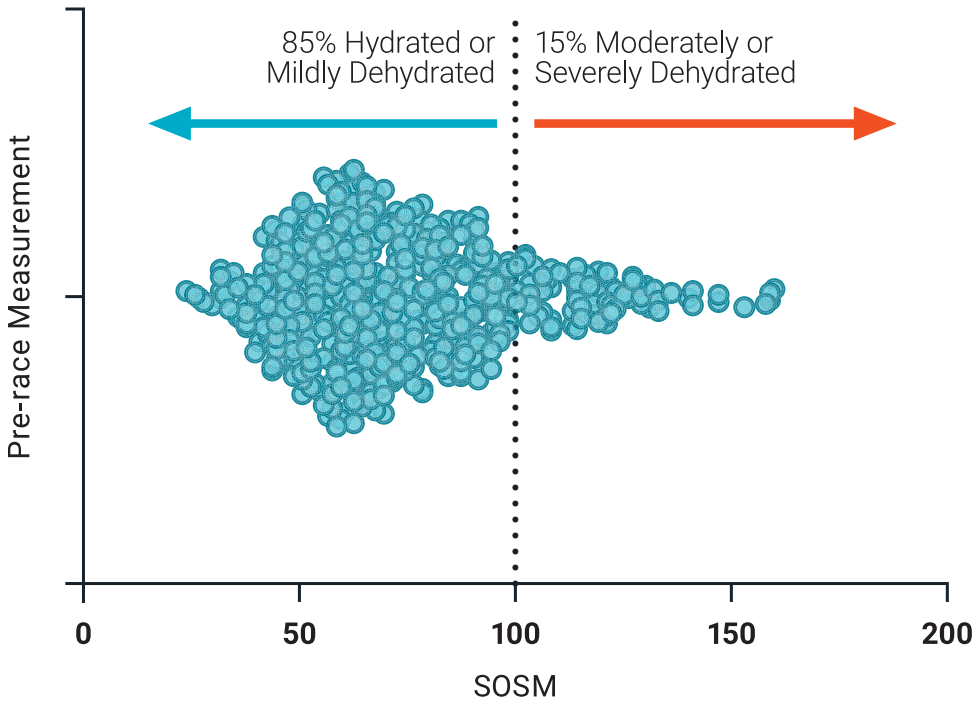


Paired measurements could be used to identify athletes who had become particularly dehydrated (SOSM > 100) and should be advised to put greater focus on rehydration during and after training.

MX3 Hydration Testing at the 2018 Melbourne Marathon

SOSM testing was offered to the public during the 2018 Melbourne Marathon Festival. Over the four-day festival, the MX3 Hydration Testing System was used to perform more than 1000 hydration measurements on over 800 athletes. Athletes were measured after only a 60-second introduction to the testing system. This event demonstrated the high-throughput capacity of the MX3 Hydration Testing System and the ease with which SOSM measurements could be collected from many different individuals. This study found that the majority of athletes (~85%) were well hydrated or only mildly dehydrated on the days leading up to the Marathon **(Figure 7)**. A subset of athletes (~15%) had a SOSM > 100 and were directed to focus on hydrating prior to and during the race to avoid the negative effects of dehydration on performance.

Figure 7 - Distribution of SOSM measurements taken on the days leading up to the 2018 Melbourne Marathon Festival (n=493)



SOSM Measurements were used to identify athletes at greater risk of dehydration and provide hydration advice.

References

1 Casa, D. J. et al. National athletic trainers' association position statement: fluid replacement for athletes. J Athl Train 35, 212-224 (2000).

2 Baker, L. B. & Jeukendrup, A. E. Optimal composition of fluid-replacement beverages. Compr Physiol 4, 575-620, doi:10.1002/cphy.c130014 (2014).

3 McDermott, B. P. et al. National Athletic Trainers' Association Position Statement: Fluid Replacement for the Physically Active. J Athl Train 52, 877-895, doi:10.4085/1062-6050-52.9.02 (2017).

4 Sommerfield, L. M. et al. Validity of Urine Specific Gravity When Compared With Plasma Osmolality as a Measure of Hydration Status in Male and Female NCAA Collegiate Athletes. J Strength Cond Res 30, 2219-2225, doi:10.1519/JSC.0000000000001313 (2016).

5 Hew-Butler, T. D., Eskin, C., Bickham, J., Rusnak, M. & VanderMeulen, M. Dehydration is how you define it: comparison of 318 blood and urine athlete spot checks. BMJ Open Sport Exerc Med 4, e000297, doi:10.1136/bmjsem-2017-000297 (2018).

6 Walsh, N. P. et al. Saliva parameters as potential indices of hydration status during acute dehydration. Med Sci Sports Exerc 36, 1535-1542 (2004).

7 Smith, D. L., Shalmiyeva, I., Deblois, J. & Winke, M. Use of salivary osmolality to assess dehydration. Prehosp Emerg Care 16, 128-135, doi:10.3109/10903127.2011.614044 (2012).

8 Munoz, C. X. et al. Assessment of hydration biomarkers including salivary osmolality during passive and active dehydration. Eur J Clin Nutr 67, 1257-1263, doi:10.1038/ejcn.2013.195 (2013).

9 Armstrong, L. E. Assessing hydration status: the elusive gold standard. J Am Coll Nutr 26, 575S-584S (2007).

10 Stookey, J. D., Hamer, J. & Killilea, D. W. Change in hydration indices associated with an increase in total water intake of more than 0.5 L/day, sustained over 4 weeks, in healthy young men with initial total water intake below 2 L/day. Physiol Rep 5, doi:10.14814/phy2.13356 (2017).