White Paper: Use of the MX3 Hydration Testing System for hydration assessment

This document outlines the evidence behind the use of Salivary Osmolarity (SOSM), as measured by the MX3 Hydration Testing System (HTS), for hydration assessments in various applications.

Table of Contents

Version Number	Revision Date
Version 1.2	07/02/2022

1. Medical Disclaimer

The MX3 Hydration Testing System is not a medical device. The MX3 Hydration Score and MX3 Hydration Categories are not medical advice and are not intended to diagnose, cure, mitigate, treat, or prevent any disease or health condition. MX3 Hydration Categories are guidelines only and may vary depending on the individual.

If you or the individual being measured are experiencing headaches, nausea, or other symptoms of dehydration or heat strain before, during, or after work or exercise, please seek immediate medical attention.

2. The importance of hydration

Occupational Health and Safety

Adequate and regular fluid and electrolyte intake is essential for offsetting fluid losses when working in the heat. Where fluid losses are not appropriately replaced, dehydration can result in cognitive and physical impairment[1].

A decrease in body water content can also reduce sweat rate. As evaporative cooling from sweating is the primary mechanism for thermal regulation when working in the heat, dehydration can result in a reduced ability for the body to regulate temperature, resulting in an increased risk of heat strain[2].

Hydration alone may not be sufficient to avoid heat strain when working in extreme conditions where evaporative cooling is impaired or insufficient, such as very high temperature, high humidity and/or low airflow environments as well as when heavy protective equipment is being worn[3].

While fluid replacement is key for avoiding heat strain, hydration alone is not sufficient to avoid heat strain in all contexts and should be used in combination with appropriate heat stress management, acclimation, and control programs.

Version Number	Revision Date
Version 1.2	07/02/2022

Sports and Fitness

Fluid loss, including water and electrolytes, during exercise of greater than 1% bodyweight begins to impact cognition and decision making[4] . Fluid loss greater than 2% results in reduced muscular strength and endurance and increases the risk of heat-related injury[5].The higher the degree of dehydration, the more pronounced and consistent the impact on physical and mental performance

While a certain amount of fluid loss is expected during training or competition, if an athlete begins exercising in a fluid deficit, dehydration and reduced performance will occur faster, with chances of injury increasing with duration of exercise. Therefore, managing the hydration of athletes so that they start training or competition fully hydrated can help maintain performance by providing a buffer to the adverse effects of exercise related dehydration[6].

Tracking hydration for an athlete is a continuous challenge, so pre-exercise preparation needs to be coupled with adequate and appropriate hydration during exercise and effective rehydration and recovery post-exercise.

Military and Defense

In addition to the occupational health and safety risks described above, which are of relevance for military groups during training and deployment[7], the impact of fluid deficit on physical exertion can significantly reduce physical and mental performance and potentially compromise mission safety and objectives[8,9].

Dehydration as little as 2% body mass loss (BML) causes significant performance deficits and much greater dehydration - up to 6% BML - has been reported in short duration (<125mins) training flights and missions[10]. At this level of dehydration there is a great risk of heat exhaustion, heat stroke, loss of consciousness, and organ damage[6].

Monitoring hydration can guide warfighters to be optimally hydrated prior to missions, as well as effectively rehydrate and recover. This may improve performance and the likelihood of meeting mission objectives as well as overall mission safety.

3. Evidence for the use of SOSM as a hydration assessment biomarker

Salivary osmolarity (SOSM) is the number of solute particles per litre saliva. In some contexts, salivary osmolality, the number of solute particles per kg of saliva, is reported instead. Given that the relative density of saliva is <1.01[11], these terms may effectively be used interchangeably.

Appendix 1 details the literature investigating the use of SOSM for hydration assessment, where SOSM is benchmarked against plasma osmolarity or changes in nude body mass (BML) or other gold standard measures of dehydration. Broadly speaking, these studies can be summarised as follows:

- Most studies investigating SOSM as a hydration biomarker have been conducted in populations of young and healthy adults, observing changes in SOSM before, during and after active (physical activity-induced) dehydration, or looking at various methods of passive dehydration such as extended heat exposure, fluid restriction, and chemical induced sweating.

- In general, SOSM demonstrates a greater responsiveness to physical activity-induced dehydration than passive dehydration, serving as an effective indicator of fluid loss with regard to nude body mass loss. Where these studies have also integrated urinary measures, SOSM typically has superior or equivalent performance in active dehydration contexts.

- Sports and Fitness. Most evidence for SOSM as a hydration biomarker has been collected in a sports science context. Specific studies which investigate SOSM in exercise-related contexts of various intensity and have demonstrated utility for tracking hydration include Walsh (2004)[12], Oliver (2008)[13], Munoz (2013)[14] and Kitson (2021)[15].

- Workplace Hydration Monitoring: Several studies have specifically examined SOSM measurements a context intended to simulate a strenuous work environment. In one study, changes in SOSM during active dehydration were assessed during extended exercise trials while wearing either light clothing or heavy protective equipment[16]. Changes in SOSM were strongly correlated with percentage body mass loss, with a significantly higher SOSM observed when wearing heavy protective equipment. In the same cohort, change in body mass was not highly correlated with change in urinary osmolality. Another study investigating changes in SOSM and urine parameters in firefighters undergoing training in full structural firefighting bunker-style gear and a self-contained breathing apparatus demonstrated a significant increase in SOSM before and after training which significantly correlated with change in body mass, while no changes in urine parameters was observed[17].

- SOSM has been investigated in a clinical context, specifically, assessment of hydration in older adults presenting to the emergency department[18]. In this context, SOSM was reported as the most sensitive and specific method for diagnosing dehydration out of a panel of physical signs, urinary biomarkers, and salivary biomarkers, when benchmarked against plasma measures of hydration status.

Like all field measures of hydration status, SOSM has its limitations.

However, on balance, SOSM has been reported to be an effective compromise between accuracy and ease-of-use in active dehydration contexts where gold-standard measures such as plasma osmolarity are not practicable.

4. Limitations of SOSM hydration assessments and how these are addressed by MX3

Several limitations associated with SOSM have been reported[14,19]. During the development of the MX3 HTS we sought to address these limitations or reduce the impact they have on hydration measures.

There may be significant inter- and intra-individual variation in SOSM values, and characterisation of this variation may require assessment across multiple days.
Due to the cost and complexity of laboratory-based osmometers it has been difficult to generate a measurement dataset for effectively characterisation of variation in SOSM. Due to the rapid and accessible nature of the MX3 HTS it is much more achievable to collect this dataset. Using the "Baseline" feature of the MX3 Mobile App (see the MX3 HTS User Manual), an individual's typical SOSM range in a well hydrated state can be measured by the MX3 HTS and used to personalise hydration categories to allow for more individualised hydration assessment.

Where baselining is not practicable, comparison to populational reference ranges is still useful given the typically large changes in SOSM which are observed during moderate or severe dehydration compared to typical variation in hydrated individuals.

Sample collection can be difficult after strenuous physical activity, where it is difficult to generate a large volume of saliva. Additionally, some individuals may naturally have more viscous saliva independent of hydration status.
Unlike conventional osmometers which require 10s or 100s of microliters of saliva for a SOSM measurement, the MX3 HTS requires less than a single microlitre. This allows for measurement in the context where only a small amount of saliva can be generated. Additionally, MX3's patented salivary test strip microfluidics is designed to accommodate even highly viscous saliva.

Recent food or drink intake can interfere with measurements.
As water typically has a very low osmolarity, a measurement made immediately after drinking water may result in a very low SOSM. Other food and beverages, such as high salt sports drinks, have a quite high osmolarity (300+) so a measurement made immediately after drinking or eating may result in an artificially increased SOSM.

To avoid this confounding factor, we have a set of measurement instructions (see example protocol below) which serve to minimise the potential impact of food or drink, including the production of fresh saliva for analysis and a recommendation to wait at least 5 minutes between eating/drinking and performing an MX3 measurement.

To help ensure this suggestion is implemented, users can be asked to make a declaration that they did not eat/drink in the last 5 minutes when taking a measurement using the MX3 App. Additionally, abnormal SOSM measures are detected with specialised error detection algorithms and reported to organisation administrators though the MX3 Web Portal to highlight misuse of the MX3 HTS.

5. Analytical Performance of The MX3 Hydration Testing System (HTS)

Two independent studies have benchmarked the MX3 HTS against a conventional laboratory freezing-point depression osmometer on a panel of saliva samples. Both studies reported an excellent correlation between MX3 HTS reported SOSM values and values measured by an osmometer (Study 1[20]: R^2 = 0.95 **Figure 1**, Study 2[21]: R^2 = 0.92,). Replicate readings of collected saliva samples have a typical CV of approximately 6±2% (table 1).

Serial measurement of saliva sampled directly from the tongue will typically exhibit a larger amount of variation due to the confounding effects of sampling variation. As each MX3 measurement only uses 1 microliter of saliva, the precise composition of this sample will vary depending on the contribution of various salivary glands. In these contexts, a higher CV of 10±5% is expected, and measurements may vary by up to 25%. Where a more precise measurement is required, a larger sample should be collected in an appropriate sampling receptacle, for example an MX3 sampling tray, and mixed prior to measurement.

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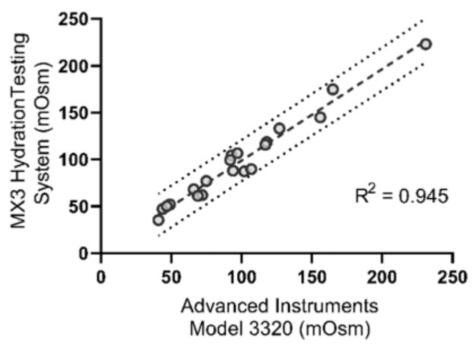

Figure 1: Correlation of SOSM readings for 20 saliva samples measured with the MX3 Hydration Testing System (average of triplicate measurement, each replicate used to calculate correlation coefficient) and an Advanced Instruments Model 3320 laboratory osmometer (single measurement). Dotted lines represent 95% prediction bands.

Table 1: Precision and Accuracy of 3 Hydration Test Strip Production Batches

	Batch 1	Batch 2	Batch 3
Validation Samples (n)	10	20	21
Correlation with Reference Osmometer (R^2)	0.95	0.96	0.98
SOSM range	42 to 205	40 to 202	45 to 201
Slope	0.94	0.92	1.08
Intercept	0.4	3.0	-6.3
Average CV (Replicate Saliva Readings)	4.8 ± 1.1%	5.7 ± 2.4%	6.8± 2.0

Version Number	Revision Date
Version 1.2	07/02/2022

6. Hydration Classification with the MX3 HTS

The MX3 HTS reports both salivary osmolarity values in milliosmoles (mOsm) and a hydration classification into four categories; Hydrated (<66 mOsm) Mildly Dehydrated (66-100 mOsm), Moderately Dehydrated (101-150 mOsm) and Severely Dehydrated (151+ mOsm).

MX3 hydration classifications are not medical advice and are not intended to diagnose, cure, mitigate, treat, or prevent any disease or health condition or to monitor or modify any physiological process.

These classifications are indications of hydration status derived from literature reports of salivary osmolarity and the observed distribution of SOSM values in the general population. They are intended to assist in the identification of individuals with an abnormal SOSM value for further investigation. Both health conditions and medication may impact SOSM readings (see section 7 below).

Figure 2 shows a distribution of over 250,000 SOSM measurements conducted by our customers. Approximately 66% of measurements are below 65 mOsm, 92% of measurements are below 100 mOsm and 99% of measurements are below 150 mOsm during regular use.

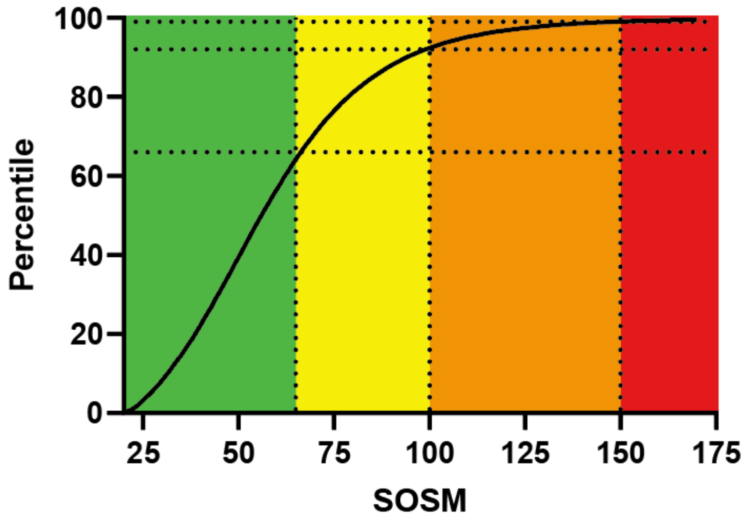

Figure 2: Distribution of >250,000 MX3 SOSM measurements conducted between Jan 2019 and Dec 2021. Colours represent default MX3 HTS hydration categories.

Version Number	Revision Date
Version 1.2	07/02/2022

7. Impact of health conditions and medication on SOSM values

Like all biomarkers in bodily fluids, SOSM measurements may be impacted by medication or underlying health conditions.

While there are no published reports demonstrating the influence of specific medications and health conditions on the use of SOSM measurements as a hydration indicator, there is a significant body of research reporting the impact of medication and health conditions on the incidence of Xerostomia (dry mouth) as well as Sialorrhea (drooling or excess salivation).

As such, these medications and health conditions may impact salivary flow rate and increase or decrease SOSM readings. In addition, other medications that target changes in fluid balance for therapeutic benefit (e.g., diuretics) may also influence SOSM. Certain medical conditions, including viral infections, diabetes, and other autoimmune disorders, can also increase the incidence of Xerostomia. Various neurological and oral conditions have also been associated increase saliva secretion.

A detailed list of medications and health conditions that can cause Xerostomia or Sialorrhea can be found in Mortazavi et al. 2014[22], Miranda-Rius et al. 2015[23], and Wolff et al., 2016[24].

If MX3 measurements are to be undertaken in individuals with existing health conditions and/or who are on medications that may impact salivary flow (and therefore SOSM), we recommend the following actions to ensure consistent and relevant sampling and measurement data:

- Baseline values are recorded for individuals when they are fully hydrated to generate a personalised SOSM reference range for hydration assessment that will be more relevant to their health/medication status. This can be carried out through a baselining protocol, such as the one outlined in the MX3 HTS manual.
- Prior to measurements ensure the user swallows existing saliva in their mouth and generates a fresh saliva sample. In the case of excessive dry mouth (Xerostomia), ask the user to consume a small amount of water and wait 5 minutes prior to measurement.
- When medication dose is changed, or additional medications are added to any regimen we recommend re-baselining the SOSM range for the individual to ensure accurate hydration status assessment. This can be done by deleting all baseline measurements within a user's MX3 profile and replacing these with new baseline SOSM values.

8. Comparison of SOSM and Urine Specific Gravity (USG) measurements

USG spot-check measurements (testing of samples other than well-controlled first-morning voids) are common practice for many organizations and sports teams as a tool for hydration assessment. As such, USG measurements are often proposed as the reference method for hydration status when looking to validate the performance of the MX3 HTS for hydration assessment.

Several recent studies have cast doubt on the appropriateness of USG spot-checks as an indicator of hydration status due to many confounding factors, including the influence of physical activity, diet and fluid intake on urine production and concentration[25-28]. These studies have all demonstrated that USG spot-checks have a very poor specificity, meaning that upwards of half of hydrated workers could be incorrectly classified as dehydrated when using this method. USG measurements using first morning voids are the only validated urine measurements that can, in the absence of underlying health conditions and medications, be used to indicate hydration status.

The scientific literature has shown USG spot checks are unreliable. MX3 does not encourage the comparison of MX3 SOSM values with Urine Specific Gravity (USG) spot-checks (testing of samples other than well-controlled first-morning voids).

Our own internal studies have found good correlation ($R^2 = 0.55$) between SOSM measurement and first-morning void USG (Figure 3).

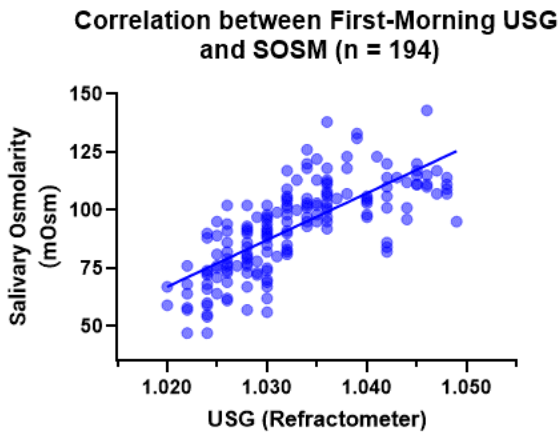

Figure 3: Correlation of first-morning void USG (refractometer) and Salivary Osmolarity in a population of 194 older adults with hypertension ($R^2 = 0.55$)

When looking to validate the performance of MX3 measurements a more appropriate comparison is between MX3 SOSM measurements and nude body mass loss (BML) during active dehydration. Plasma osmolarity, considered to be a gold standard for water loss dehydration, is also an appropriate comparator, but will not likely be accessible in a workplace or sport environment.

If you wish to run a validation study and would like some technical guidance, please contact MX3 Support (support@mx3diagnostics.com)

Version Number	Revision Date
Version 1.2	07/02/2022

9. Example protocol for the measurement of SOSM

Before measurement

1. Ensure all individuals operating the MX3 HTS have received training on how to operate the MX3 HTS, sample saliva, identify confounding factors, and interpret SOSM measurement results (see below).
2. Confirm with the individual being measured (the 'user') that they have not ingested any food or fluids in at least 5 minutes.

Preforming an MX3 SOSM Measurement

1. Log into the MX3 App and select the user's profile.
    - Do not use one user account to record measurements for multiple users. Each measurement should be linked to a single user.
    - Do not have multiple operators use a single account to make measurements. Each operator should use an independent account. For an organisation this can be achieved through assigning Admin rights to operators from the main parent account.
2. Follow the prompts in the MX3 App, instructing you to turn on the MX3 LAB (where required), sync the MX3 LAB (where required) to transfer local measurements to the App and insert a hydration test strip.
    - Optional: Have the user confirm they have not ingested any food or fluid in the last 5 minutes using the "Food/Drink confirmation" feature of the MX3 App.
3. Ask the user to swallow all saliva in their mouth, then to generate a fresh saliva sample (as if they were going to spit) and position the sample on their tongue.
4. Ask the user to stick out their tongue. Ensure a good amount of saliva is present on the user's tongue. The tongue should be noticeably wet.
    - If insufficient saliva is present, ask the user to swallow and generate a fresh sample.
    - If the saliva sample is highly bubbly, ask the user to swallow and generate a fresh sample.
    - If food debris are present in the saliva, ask the user to rinse their mount with water and return for a measurement in 5 minutes.
5. Tap the tip of the test strip against the saliva sample on a downward angle until the MX3 LAB beeps, indicating that enough saliva has been collected.
6. Wait for the sample to be analysed, you will hear a second beep when completed
    - Do not remove the test strip during this period.
7. The result will then be displayed in the MX3 App together with the hydration classification.
8. Eject the hydration test strip into a waste container using the eject button.

10. Interpretation of MX3 measurements

MX3 hydration classifications are not medical advice and are not intended to diagnose, cure, mitigate, treat, or prevent any disease or health condition or to monitor or modify any physiological process. Where a user is experiencing any symptoms of heat strain or dehydration such as prickly heat, fatigue, nausea, or dizziness these individuals should refrain from further physical activity and seek medical attention.

Measurement interpretation will be specific to your environment and should be considered in combination with other heat stress and dehydration indicators.

The interpretative guidance given below may be used as a starting point for developing interpretive guidelines for your specific context.

Hydrated

Before Work/Exercise: This score indicates that the user is in a well-hydrated state and regularly consuming enough fluid to prepare for work or exercise.

After Work/Exercise: This score indicates that the user has appropriately hydrated during work or exercise. However, during intense and extended physical activity it is unlikely that all fluid losses would have been mitigated and therefore the user should eat and drink as normal to assist rehydration and recovery.

Mildly Dehydrated

Before Work/Exercise: This score indicates the user is slightly dehydrated and should increase their regular fluid intake to better prepare for work or exercise. They should have an additional drink before starting work or exercise and maintain consistent hydration throughout their shift.

After Work/Exercise: This score indicates that the user is slightly dehydrated. This is a normal score after work or exercise. Eating and drinking as normal over the next few hours should allow the user to fully rehydrate and recover.

Moderately Dehydrated

Before Work/Exercise: This score indicates the user is quite dehydrated. They should significantly increase regular fluid per day to better prepare for work or exercise and actively drink additional fluids before starting work or exercise to minimize further dehydration.

After Work/Exercise: This score indicates the user is quite dehydrated. They should actively consume fluids over the next few hours to assist rehydration and recovery. As a guide we recommend consuming between 6-800ml per hour. In future this user should look to increase fluid intake during work or exercise to offset fluid losses.

Severely Dehydrated

Before Work/Exercise: This score indicates the user is significantly dehydrated. This is an unusual result before work or exercise and suggests they have not fully recovered from prior dehydration. If possible, the user should refrain from work or exercise until they have had the opportunity to rehydrate. Further indicators of heat stress and fatigue should be assessed to ensure user safety.

Before Work/Exercise: This score indicates the user is significantly dehydrated. They should actively consume fluids over the next few hours to assist rehydration and recovery. As a guide we recommend consuming between 6-800ml per hour. In future this user should look to increase fluid intake consistently during work or exercise. The user should avoid further activity and discuss hydration strategies with their coach or supervisor.

Appendix 1: Peer-Reviewed studies investigating SOSM and hydration status

This table contains a chronological list of peer-reviewed studies that investigate Salivary Osmolarity and Hydration Status. To be included on this list SOSM must be directly compared with either plasma osmolarity or changes in body mass. Where urine measurements were also performed this has been noted.

Study	Population	Summary
Walsh, 2004	15 volunteers All Male, Age = 23 ± 3	Link: https://journals.lww.com/acsm-msse/Fulltext/2004/09000/Saliva_Parameters_as_Potential_Indices_of.12.aspx Study: SOSM and UOSM were benchmarked against changes in body mass and POSM during active dehydration with and without fluid replacement Outcome: • During exercise with fluid restriction SOSM increased from 50 ± 11 mOsm to 105 ± 41 mOsm at 3% BML. • No change in SOSM was observed during the fluid replacement trial. • SOSM strongly correlated with plasma osmolarity (r = 0.87) • SOSM remained unchanged immediately or 15 minutes after consuming a beverage (data not published) Urine Comparison: in this study UOSM was also shown to increase during fluid restriction, but several participants were unable to provide a urine sample at 2.1 and 3% BML. UOSM was significantly lower during the fluid intake trial when compared to pre-exercise. UOSM strongly correlated with plasma osmolarity (r = 0.83)
Oliver, 2008	13 volunteers All Male Age = 21 ± 1	Link: https://www.sciencedirect.com/science/article/abs/pii/S0003996908001453?via%3Dihub Study: Investigation of the responsiveness of SOSM measures to normal fluid intake (CON), fluid restriction (RF) across 48 hours and fluid and energy restriction (RF+RE) across 48 hours as well as during an active dehydration and rehydration trial. Outcome: • At 48 h body mass loss exceeded 3% on RF and RF + RE. • SOSM increased during 48 h on RF (54 ± 3 to 73 ± 5 mOsm and RF + RE (52 ± 3 to 68 ± 5 mOSM) and was significantly greater than CON at 48 h (52 ± 2 mOsm) • SOSM identified the additional dehydration associated with exercise and returned to within 0 h values with rehydration • Given the large amount of variation between individuals, the authors recommended that a euhydrated reading is determined for saliva markers for each individual. Urine Comparison: Urine osmolarity used to verify hydration status at the start of each protocol but was not tracked during the various protocols.

Version Number	Revision Date
Version 1.2	07/02/2022

Cheuvront, 2010	18 Soldiers 13M, 5F Age = 24±4	**Link:** https://academic.oup.com/ajcn/article/92/3/565/4597438 **Study:** Comparison of plasma, urine, and saliva biomarkers for static (euhydrated) and dynamic (active dehydration benchmarked against body mass loss) hydration assessment **Outcome:** • Plasma osmolarity was the only body fluid that was considered to have appropriate inter- and intra- individual variation to provide a correct diagnosis of dehydration from a static individual value. SOSM displayed an approximate 10% within-subject CV. • SOSM displayed an 80% sensitivity and 83% specificity for identifying dynamic dehydration with a threshold value of 83 mOsm. SOSM values increased from 88±32 mOsm in the hydrated group to 147 ± 88 mOsm after dehydration. • SOSM measurements conducted 1 minute after a water mouth rinse dropped to 81 ± 25 mOSM even though volunteers were still dehydrated – highlighting the need for food/drink restriction prior to measurement. **Urine Comparison:** In this study urinary markers were also considered to be inappropriate for static (euhydrated) assessment due to large amounts of inter- and intra-individual variation. USG outperformed SOSM with a sensitivity of 89% and a specificity of 91% using a threshold value of 1.025 units.
Ely, 2011	8 healthy volunteers 6M, 2F Age = 22±7	**Link:** https://journals.lww.com/acsm-msse/Fulltext/2011/06000/Limitations_of_Salivary_Osmolality_as_a_Marker_of.21.aspx **Study:** POSM, SOSM and USG were benchmarked against changes in body mass during active dehydration following a 3-day euhydration protocol. **Outcome:** • SOSM displayed a within-subject CV of 6.6% • SOSM increased from 58±8 mOsm to 96±28mOsm after active dehydration. • SOSM had a correlation of r = -0.71 with change in body mass, while POSM had a correlation of r = -0.87 • Saliva osmolarity decreased after an oral rinse but returned to pre-rinse values after 15 minutes and remained similar at 30 minutes. **Urine Comparison:** In this study USG was shown to increase from 1.018 ± 0.005 during euhydration to 1.028 ± after active dehydration. The correlation between USG and changes in body mass was not reported.
Smith, 2011	8 Healthy volunteers All Male Age = 24±5	**Link:** https://www.tandfonline.com/doi/abs/10.3109/10903127.2011.614044 **Study:** POSM, SOSM and UOSM compared with BML during two exercise trials, one wearing shorts and t-shirt (EX), the other wearing firefighting personal protective equipment (EX+PPE) **Outcome:**

		• POSM did not significantly increase during either trial, despite a 0.81±0.3% BML during EX and 2.18±0.54% BML during EX+PPE • SOSM increased significantly in both trials, 73.4 ± 12.4 to 125.1 ± 30.3 mOsm (EX+PPE) and 70.1 ± 12.5 to 83.6 ± 17.7 mOsm (EX); with the increase in the EX+PPE trial significantly greater than the increase in the EX trial. • Changes in salivary osmolality were strongly correlated with percent BML (r = 0.80) Urine Comparison: Urine osmolality increased significantly from pre to postexercise time points in both trials, but there was no significant difference between trials.
Horn, 2012	35 firefighters 31M/4F Age = 35±10	Link: https://www.tandfonline.com/doi/abs/10.3109/10903127.2012.664243?journalCode=ipec20 Study: SOSM, BML and Urine measures measured before and after 3 hours of firefighter training exercise. Participants wore full structural firefighting bunker-style gear and a self-contained breathing apparatus (SCBA) during all firefighting activities Outcome: • Participants lost an average of 1.1 ± 0.8 kg (1.4%) of body mass ($p < 0.001$). During the firefighting activity, 63% lost more than 1% of their body mass, 26% lost more than 2% of their body mass, 9% lost more than 3% of their body mass. • There was a significant increase in SOSM after firefighting exercises ($p<0.001$) • There was a significant correlation of r = 0.56 (p = 0.001) between changes in body mass and the salivary osmolality measures. This correlation improved to r = 0.62 (p = 0.002) when considering only participants who showed a change in body mass of greater than or equal to 1%, while there was no correlation for firefighters who experienced less than 1% change in body mass(r = 0.05). Urine Comparison: No significant changes in urinary measurements were detected between pre- and post-firefighting values
Taylor, 2012	12 volunteers All Male Age not reported	Link: https://link.springer.com/article/10.1007%2Fs00421-011-2299-z Study: SOSM and BML tracked during three exercise and heat induced dehydration trials (7%, 3% and 7% dehydration). Outcome: • SOSM increased linearly during dehydration, though baseline and rate of change varied among and within subjects between trials, with an average correlation for each individual of r = 0.81, r = 0.5 and r = 0.70 for trials 1, 2 and 3 respectively. • Looking across all data, SOSM was a sensitive and specific discriminator of >3% or >6% binary dehydration classification (sensitivity and specificity ~ 80%) but not ineffective for discriminating between 3-6% and 6%+

		• Given the inter-individual variability the authors highlight the need for a pre-exposure baseline SOSM measures when attempting to perform more fine-grain hydration assessments. Urine Comparison: USG was used to verify hydration status at the start of each protocol but was not tracked during the various protocols.
Munoz, 2013	23 volunteers All Male Age = 22±3	Link: https://www.nature.com/articles/ejcn2013195 Study: Measurement of body mass change, SOSM, UOSM, USG during 5 hours of passive heat exposure (PAS) and active exercise (ACT) in the heat. Outcome: • While an average BML of 1.4± 0.3% was observed during PAS, neither SOSM of POSM was significantly elevated • During ACT SOSM most effectively diagnosed dehydration ⩾2% (sensitivity=86%; specificity=91%), followed by POSM (sensitivity=83%; specificity=83%). • ~35mOSM increases in SOSM were observed per percentage BML during active dehydration. SOSM increased from 64 ± 9 before active dehydration to 90 ± 24 at 1% BML. Urine Comparison: In this study USG was shown to be an effective indication of mild dehydration during passive heat exposure, with a significant increase at 1% BML. USG was also an effective hydration indicator during active dehydration, performing slightly worse that SOSM with a sensitivity of 81% and a specificity of 81%.
Perrier 2013	52 adults 11M, 41F Age = 25 ± 3	Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3714557/ Study: Measurement of urine (accumulated AM/PM collection), SOSM (every 1-2 hours) and POSM (AM/PM) across an inpatient cross-over trial. Condition 1: fluid restriction across 2 days, Condition 2: Euhydration. Outcome: • No difference was observed in SOSM between water intake conditions. No difference in POSM was observed as well. • SOSM varied throughout the day, but at no point averaged over 95 mOsm or below 75 mOsm. Drops of ~10 mOsm were observed ~ 30 minutes after each meal. Urine Comparison: Urinary hydration biomarkers were responsive to changes in water intake, but the extended collection method makes it unclear how representative spot-check measurements would be in this context.
Pross 2013	20 healthy adults All Female Aged = 25± 1	Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3553795/ Study: Measurement of USG, POSM and SOSOM during 24 hours of fluid restriction vs fully hydrated control condition • No change in POSM was observed during fluid restriction. • A small but significant SOSM increase was observed after 24 hours fluid restriction (57.3 vs 68.6 mOsm) Urine Comparison: USG was significantly elevated at all timepoints during fluid restriction,

Ely, 2014	24 healthy volunteers 17M, 7F Age = 23 ± 4	**Link**: https://link.springer.com/article/10.1007%2Fs00421-013-2747-z **Study**: Paired euhydration and dehydration trails. Dehydration achieved using furosemide (diuretic) administration and 12 hour fluid restriction (extracellular dehydration) **Outcome:** • SOSM changes were marginal (<10 mmol/kg) and weakly correlated with changes in absolute or relative plasma volume losses • Strong agreement was observed between SOSM collection methods (Expectoration: 61 ± 10 mOsm, Salivette: 61 ± 8 mOsm. **Urine Comparison** • Extracellular dehydration was also not detectable using USG.
Fortes, 2015	130 Older Adults 59M, 71F Age = 79±9	**Link:** https://www.jamda.com/article/S1525-8610(14)00614-8/fulltext **Study:** Comparison of physical signs, urinary biomarkers, and salivary biomarkers against plasma osmolarity and BUN/Creatinine ratio when presenting to a hospital emergency department. **Outcome:** • SOSM demonstrated superior diagnostic accuracy when compared with physical signs and urine markers and was able to detect both water-loss and water-and-solute loss dehydration. • Water-loss dehydration: 70% sensitivity, 68% specificity, OR = 5.0 • water-and-solute-loss dehydration: 78% sensitivity, 72% specificity, OR = 8.9 **Urine Comparison:** In this study neither USG nor urine colour were able to discriminate between dehydration and euhydration
Stookey, 2017	5 Health Adults All Male Age: 20-25	**Link:** https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5704074/ **Study:** Investigation of various hydration indices associated with an increase in total water intake across 6 weeks in individuals with chronic dehydration. **Outcome:** • Body weight increased significantly by a mean ± SEM of 1.8% ± 0.5% from baseline over 4 weeks of increased fluid intake and SOSM decreased significantly • Baseline saliva osmolality significantly modified responses to chronic water intake • Saliva osmolality decreased significantly between Week 1 and Week 6 by an average ± SEM of 4 ± 1 mmol/kg per week • Only the saliva osmolality classification predicted significantly modified responses to sustained higher water intake over subsequent weeks.

		Urine Comparison: Urine sodium, potassium, and osmolality decreased significantly by an average ± SEM of 11 ± 4 mmol/L, 8 ± 2 mmol/L, and 85 ± 15 mmol/kg per week, respectively. "The results of this study suggest that saliva osmolality may be a more sensitive and specific indicator of change in chronic total body water than serum or urine osmolality. At baseline, only the saliva osmolality classification varied between participants."
Dulson, 2019	12 Endurance athletes All Male 29± 3	**Link:** https://onlinelibrary.wiley.com/doi/full/10.1002/tsm2.73 **Study:** Investigating the impact of caffeine ingestion on various salivary biomarkers during exercise **Outcome:** • Participants lost ~2.5% of their body mass in fluid loss over the course of a 70 minute intense run. • Over the course of the run SOSM increased from ~55mOsm to ~75 mOsm mid-exercise and ~85mOSm post-exercise. 1 hour post exercise where participants we provided water to rehydrate SOSM had retured to ~55mOsm. (exact values for whole cohort were not reported). • Salivary osmolarity was significantly higher mid- and post-exercise compared to pre-exercise and post-recovery (P< 0.0001) and significantly higher post-exercise than mid-exercise (P<0.01) **Urine Comparison:** Urine samples were not analysed in this study.
Harris, 2019	17 participants 9M, 7F Age 20-25	**Link:** https://jissn.biomedcentral.com/articles/10.1186/s12970-019-0282-y **Study:** Investigating the effect of various beverages on the recovery from 3% BML caused by active dehydration **Outcome:** • SOSM significantly increased (p < 0.0001) with loss of body mass during the dehydrating exercise protocol. • Baseline SOSM was not affected by sex in the stimulated (females 94.39 ± 14.90 vs males 113.00 ± 63.84), or the unstimulated (females 94.06 ± 26.62 vs males 95.51 ± 33.27) saliva samples, • Peak SOSM was not significantly impacted by either study group designation or sex in the stimulated (females 180.29 ± 60.37 vs males 256.96 ± 104.57), or the unstimulated (females 235.04 ± 105.99 vs males 297.14 ± 102.39) saliva samples. • At the completion of 3% body mass loss, all participants achieved a significant (p < 0.0001) increase in averaged SOSM over baseline values **Urine comparison:** Urine was not collected in this study
Owen, 2019	15 healthy volunteers All Male Age = 23 ± 5	**Link:** https://journals.humankinetics.com/view/journals/ijsnem/29/6/article-p604.xml **Study:** comparison of physical signs and symptoms, urinary, salivary and tear biomarker for identifying mild intracellular (fluid restriction) and extracellular dehydration (chemical induced). **Outcome:** • SOSM significantly increased during intracellular dehydration (56 ± 12 mOsm to 64 ± 13 mOsm) but did not increase during extracellular dehydration

		• Urine colour, urine specific gravity, plasma osmolality, saliva flow rate, saliva osmolality, heart rate variability and dry mouth identified mild fluid restriction induced dehydration (ROC-AUC; range 0.70-0.99) Urine Comparison: USG and UOSM were superior predictors of intracellular dehydration due to fluid restriction, with a ROC AUC of 0.99 (SOSM = 0.7)
Kitson 2021	27 healthy volunteers M = 13, F = 14 Age = 25 ± 8	Link: https://www.mdpi.com/2072-6643/13/10/3313 Study: Study investigating the perception of various rehydration beverages when exercising in the heat. SOSM, USG and BML measured at various timepoints during exercise. Outcome: • Body mass significantly decreased by 1.36 ± 0.39% following 60 minutes of exercise, considered by the authors to be "sub-clinical" dehydration (<2%) • SOSM gradually and consistently increased over the course of the trial from a median of 85 to a median of 113 after 60 minutes. A significant difference was observed between the 0- and 60-minute timepoints Urine Comparison USG and UOSM were measured at 0- and 60-minute timepoints. A significant change in USG was observed across the exercise trial (1.014 to 1.017) but there was no significant change in UOSM

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Version Number	Revision Date
Version 1.2	07/02/2022

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