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Effect of Urine Agitation on Measurements of Hydration Status.

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Effect of Urine Agitation on Measurements of Hydration Status

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ABSTRACT

Hypohydration can have significant implications on physiological functions of the body and has the potential to decrease level of performance. In addition to performance decrements, hypohydration can also lead to increased thermal and cardiovascular strain. As a preventative measure athletic trainers are commonly required to attain urine specimen samples to assess athlete hydration status for weight checks and monitoring body mass losses. Unfortunately, immediate examination of urine samples is not always possible. As the urine sample sits, sedimentation develops. No current literature addresses the sedimentation of urine samples and what procedures should be performed to ensure an accurate hydration assessment. OBJECTIVE: The purpose of this study is to determine if agitation of urine samples is comparable to the criterion measure, urine osmolality measured within two hours of collection. DESIGN: We used a descriptive diagnostic validity test design to investigate the effects of agitation of urine samples on the measure of hydration status. SETTING: Biochemical Research Laboratory at Indiana State University. PARTICIPANTS: Seventy-five healthy participants (41 males, 34 females; mean age=22±5years; mean self-reported height=172±23cm and mass=77±17kg) recruited from a university campus provided one or more samples (total samples=81). INTERVENTION: The independent variable was agitation type with 3 levels: vortex mixed, hand shaken, and no agitation. Following recruitment, participants completed the informed consent and a short health questionnaire to rule out any exclusion criteria such as kidney disease, diabetes, etc. Participants were provided with a clean specimen cup and were asked to provide a sample. Large samples were encouraged as they were then split evenly into three cups and labeled according to

participant number and agitation type. Hand shaken samples were shaken 10 times in an hourglass fashion, from right side up to up side down. Vortex samples were placed on the vortex mixer for 10 seconds. Non-agitation samples were not disturbed. MAIN OUTCOME MEASURES: Urine osmolality, as measured by a freezing point depression osmometer was used to determine hydration status within two hours of specimen collection and again after 48 hours. Agitation was only performed prior to the second measurement of hydration status, after 48 hours had passed. A one-way ANOVA was performed to compare the two methods of agitation against the criterion control. RESULTS: No significant differences were identified ($F_{3,316}$ = 0.00027, p =0.99, 1-β=1.00) between the no agitation (mean=724±262), hand shaken (mean=723±263) and vortex (mean=724±263) methods when compared to the criterion control (mean=723±262). CONCLUSIONS: The findings of this study demonstrated no differences in hydration status measurements between the two agitation methods and the control. For practitioners who are unable to immediately measure the hydration status of urine samples, agitation of the urine specimen is not necessary in order to obtain a valid measure of hydration status using an osmometer.

PREFACE

I have been fortunate for the opportunity to participate in scholarly work and research along side some of the most knowledgeable and passionate professors at Indiana State University.

After finishing my undergraduate education, I spent some time contemplating what area of research I wanted to focus my Master's thesis on. I was able to take Dr. Yeargin's Environmental Physiology class in my first semester of graduate school. Her enthusiasm and knowledge for heat illness research immediately intrigued me. With this new research interest, I was able to sit down with Dr. Yeargin and she recommended this project to me. This topic is relevant to all athletic trainers, researchers, and educators who have a need for urine analysis.

ACKNOWLEDGMENTS

I would like to give a special thank you to my chair and committee members for all the guidance they have provided me with over the past two years. They have made themselves available for questions and helped steer me in the right direction on multiple occasions. Andy and Dave have played an invaluable role throughout this process. Together, we spent many hours in the biochemical research laboratory analyzing more than 500 urine samples. Our research assistant, Joslyn, spent a great amount of time recording and imputing data. Additionally, I would like to thank my co-workers, Chris and Kaitlyn, and my family. The support I received from friends and family made this processes much easier.

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CHAPTER 1

INTRODUCTION

Hypohydration has the potential to affect any individual, regardless of age or activity level, as research on both youths and adults has suggested.[1-3] Maintaining a euhydrated state in the body is essential to maintaining normal bodily functions. Hypohydration can have severe implications on physiological functions of the body and has the potential to decrease the level of performance, resulting in increased fatigue and slower performance times.[4,5] In addition to performance decrements, hypohydration can lead to increased thermal and cardiovascular strain, increasing the susceptibility of life-threatening conditions such as exertional heat stroke.[6]

Numerous methods for determining hydration status exist. Hematological measures often include plasma osmolality and plasma volume shifts. Urine measures used most often include urine specific gravity (USG), urine color, and urine osmolality. Urine osmolality is considered the standard for measuring total solute concentration,[7] but USG is still considered a valid measure of hydration status.[7] Other measurement techniques used include changes in body mass, thirst scale, and 24-hour urine volume.

With such a wide array of methods available for measuring hydration status, the exact procedures that should be followed by clinicians, educators, and researchers to accurately measure the hydration status of athletes is unclear. This confusion is seen in the lack of uniformity in recommendations for hydration measurement by various institutions including the

National Athletic Trainers Association (NATA), the American College of Sports Medicine (ACSM), and the military. [6,8,9]

Development of Research Question

Current urinary specimen collection procedures are out of date and not supported by research.[10-13] It is commonly recommended to utilize the first void of the day and examine the sample as soon as possible.[12-14] Athletic trainers, health care providers, and researchers often do not have the ability to immediately examine a sample. As the urine sample sits, sedimentation develops but no current literature addresses sedimentation of samples and what procedures should be taken to ensure an accurate hydration assessment. For these situations, it would be helpful for clinicians and researchers to know if some type of agitation, such as hand shaken samples or vortex mixers, could ensure the most valid measurement of hydration status in the event that sedimentation has developed. Therefore, in urine samples, does agitation as compared to no agitation affect measurements of hydration status?

Hypothesis

- Urine assessment after agitation via the vortex will have strong, positive correlation with the criterion measure.
- Urine assessment after agitation via hand shaking will have a strong, positive correlation with the criterion measure.
- Urine assessment after no agitation and placement of the pipette directly in the sedimentation will have poor correlation with the criterion measure.
- Urine assessment after no agitation and placement of the pipette outside the sedimentation will have a moderate correlation with the criterion measure.

Conclusions

Currently, urinary specimen collection procedures are out of date and lack scientific evidence to support their recommendations.[10] Some sources recommend utilizing the first void of the day and examining the sample as soon as possible. Unfortunately, for athletic trainers, health care providers, and researchers, immediate examination of samples is not always plausible. In these instances, it would be ideal to know if some type of agitation should be used in order to ensure the most valid measurement of hydration status. Therefore the purpose of this study is to determine if agitation of urine samples is comparable to the criterion measure.

CHAPTER 2

LITERATURE REVIEW

Introduction

Hypohydration can have significant implications on active individuals. It is important for those working with active populations to be aware that hypohydration occurs, the effects it has on individuals, and how to monitor it. Therefore, this literature review will cover hypohydration, the prevalence of hypohydration, measures of hydration status, and guidelines for assessing hydration status published by well-established institutions.

Search Strategy

The CINAHL, MEDLINE, and PubMed databases were searched for the following keywords singularly or in combination; hydration, dehydration, euhydration, hypohydration, urine, osmolality, specific gravity, military, athlete, plasma volume shift. Hand searching was also used.

Hypohydration

Maintaining a steady-state condition of normal body water,[15] or euhydration, is essential for the body to maintain normal functioning. The largest effects of hypohydration are on the thermoregulatory and cardiovascular systems, both of which play key roles during physical activity.[6] It is important to maintain a euhydrated level at all times to ensure the proper functioning of these systems.

Physiologic implications from hypohydration can occur with as little as 1-2% body weight lost.[6] These small magnitudes of loss do not take long to accumulate, often in only one hour of exercise.[6] Hypohydration can have many detrimental effects to physiological responses, performance, and illnesses.

Physiological Implications

The body consists of many physiologic systems, all of which work simultaneously to maintain blood pressure, muscular function, and core body temperature. When the body is placed in a compromised state, these systems do not function as efficiently as what is necessary to maintain normal body functions.

Hypohydration of an individual can lead to significant effects on thermoregulation. The most significant effect of hypohydration during physical activity is an increase in core temperature,[6,8,15] with an additional 0.15^{o} to 0.20^{o} C for every 1% of body weight lost.[4,6] Impaired skin blood flow and altered sweating responses further add to the increase in thermal strain by delaying the onset of skin vasodilation and sweating.[6] Other affects of hypohydration on thermoregulation include decreased sweat rate, decreased maximal sweating rate, and decreased heat tolerance.[6,15] Time to exhaustion in hypohydrated individuals occurs at lower core temperatures, decreasing total performance time.[6]

In addition to increasing thermal strain, hypohydration also increases cardiovascular strain during physical activity.[6,8] This added strain on the cardiovascular system is largely caused by a decrease in stroke volume and central blood volume and an increase in heart rate, with an additional 3 to 5 beats per minute for every 1% of body mass lost.[4,6] Hypohydration can also lead to altered metabolic and central nervous system (CNS) function and increases the utilization of glycogen as an energy source.[8]

Performance Implications

Added thermal and cardiovascular strain during physical activity have implications on level of performance. Significant performance decrements can occur with minimal levels of hypohydration, as low as 2.5%.[6] Maximum aerobic power decreases with hypohydration and physical work capacity drops as much as a 35-48%.[6] A study of distance runners showed slower race times in hypohydrated individuals.[4] In addition to altered performance on physical activities, hypohydration can also lead to decreased concentration and alertness, and increased fatigue.[5]

Hypohydration and Illness

Hypohydration can leave the body more susceptible to illness. Some of the illnesses that may be affected from hypohydration include exertional heat illnesses (EHI), cold injury, high altitude illnesses, and diabetes.

In warm, humid environments evaporation can account for more than 80% of the body's heat loss.[6] In turn, any factor that can effect evaporation (dehydration, high humidity) can lead to a rise in core temperature by limiting the body's ability to dissipate heat, placing an individual at greater risk for EHI.[6,17] In a euhydrated state, the body responds to a rise in core temperature by increasing blood flow to the periphery in order to encourage heat loss.[6,15] When hypohydrated, the body is left with diminished central blood volume and must decrease skin blood flow in order to retain adequate fluids for vital organs.[6,15] As a result of diminished central blood volume, the heart must beat faster in order to make up for a loss of stroke volume, leading to increased cardiovascular strain.[6,17] The NATA has indicated dehydration may increase an athlete's risk of EHI.[18] However, there is controversy related to if dehydration directly causes

EHI. Exertional heat stroke (EHS), exertional heat exhaustion (EHE), and exertional heat cramps (EHC) are examples of EHIs in which dehydration is a risk factor.[17,18]

EHE is most commonly seen in hot, humid environments when exercise must cease due to any combination of heavy sweating, dehydration, sodium loss, or energy depletion.[18] EHS develops when the thermoregulatory system is overwhelmed and core body temperature elevates to greater than 40^{o}C.[17,18] Both EHE and EHS can present similarly and are difficult to distinguish without measuring core body temperature.[18]

Exercise-associated muscle cramps generally present themselves during or after periods of intense exercise.[18] These cramps typically lead to an acute, painful, involuntary muscle contraction that can have negative effects on an athlete's performance.[18] In addition to neuromuscular fatigue, dehydration and electrolyte imbalances are thought to be a cause of exercise-associated muscle cramps.[6,18]

When exercising in the cold, the body attempts to adapt and retain body heat by decreasing the amount of blood that is sent to the periphery.[19,20] In turn, more blood is able to remain in the central circulation of the body eventually leading to increased urine flow rates, also known as cold-induced diuresis (CID).[19,20] Over time, this increased urine output due to the cold can lead to dehydration. Hypothermia occurs when there is a decrease in core body temperature and is typically caused by the body's inability to maintain core temperature.[20] Depending on the core temperature measurement, there are varying degrees of hypothermia.[20] Frostbite occurs when there is actual freezing of body tissues.[20] Similar to hypothermia, frostbite has varying degrees of severity depending upon the depth of tissue freezing that occurs.[20] Frostbite most commonly occurs in exposed skin (nose, ears, cheeks, etc.), but is also seen in the hands and feet because of lower tissue temperatures due to peripheral vasoconstriction.[19] Current literature has

shown that dehydration does not negatively effect peripheral vasoconstriction or shivering and, therefore, does not increase susceptibility to cold injury.[20] While dehydration is not a risk factor for cold injuries, it does appear to be a symptom commonly experienced with cold exposure. In order to prevent negative performance effects during a second bout of exercise in the cold, effort should be taken to adequately re-hydrate after activity.

During high-altitude exposure, fluid losses are increased due to decreases in ambient water vapor pressure.[21] Decreased pressure at altitude leads to fluid shifts from inside the cell to the blood, resulting in diuresis.[22] Over time, decreased water volume of the ambient air will lead to more fluid loss through respiration as inhaled air must be humidified.[23] In addition, the cold temperature at high-altitude often leads to dehydration due the body's attempts to adapt by retaining body heat, leading to increased urination.[19,20,24] To make matters worse, water availability at high-altitude is relatively low with the only source being melted snow.[21] While snow may be readily available at altitude, it requires a great deal of time and energy to melt.[24] Dehydration in individuals at high-altitude is thought, by some, to be one factor responsible for high-altitude illnesses such as Acute Mountain Sickness, High Altitude Pulmonary Edema, and High Altitude Cerebral Edema.[23] While dehydration does not appear to directly cause high-altitude illnesses, maintaining adequate levels of hydration at altitude has been shown to prevent or significantly diminish acute mountain sickness and other altitude-associated illnesses.[24]

Hyperglycemia can lead to dehydration, making it important for diabetics to maintain a euhydrated state.[25,26] As blood glucose begins to rise, the kidneys filter out glucose into the urine where it is reabsorbed.[26] This glucose re-absorption has a limit and any excess is excreted out in the urine.[26] Because glucose must be dissolved in water, increasing amounts of glucose lost in the urine leads to large water losses as well.[26] If insulin is not administered and blood glucose

continues to rise, excessive water loss can lead to further dehydration in the diabetic individuals.[26]

Athletes in a hypohydrated state are more susceptible to the development of certain illnesses. Of these, some of the most commonly seen in the athletic population include exertional heat illnesses, exercise-associated muscle cramps, cold injuries, and altitude illnesses. Hypohydration in athletes with diabetes may be prone to further dehydration due to glucose's need to be dissolved in water. In order to reduce the risks of developing these illnesses, health care professionals should periodically measure the hydration status of their athletes through urine sampling in addition to educating athletes on proper hydration behavior.

Prevalence of Hypohydration

Youth

Hypohydration in youth athletes is seen often, with many initiating exercise in a hypohydrated state.[2,27,28] A study looking at the hydration status of youth soccer campers discovered at least 75% of campers were dehydrated at the onset of exercise.[1] Another study looking at the hydration status of summer football and soccer campers revealed that at baseline, 74% of all campers entered camp at least minimally dehydrated.[27] This level of hypohydration was typically maintained throughout exercise because sweat loses were adequately replaced with fluid intake during practice.[2] However, youths were unable to adequately rehydrate between exercise bouts, continually leaving them in a hypohydrated state at the onset of competition.[2] Youth athletes have been surveyed and appear to understand the implications of hydration on performance and prevention of heat illnesses.[28] Youth athletes particularly understand, after education, the assessment of hydration based on urine color and that darker urine color is an indication of dehydration.[28] In order for youths to maintain high levels of performance and

minimize risks of EHI and cold injuries, urine hydration measurements should be taken by a health care professional.

Adults

Hypohydration affects adults of many different levels of competition, including National Football League (NFL), Division I Intercollegiate (DI), Division II Intercollegiate (DII), military, and distance runners. With high caliber athletes, even the smallest performance decrements could make the difference between winning and loosing. In order to catch hypohydration before it leads to performance decrements or illness, hydration status should be measured.

Mild dehydration has been observed in NFL players in a study looking at body weight changes during two-a-day preseason football practices.[29] Not only NFL athletes are at risk, as one study looking at the hydration status of DI football players discovered significantly increased urine color, increased urine specific gravity (USG), and mild body weight losses pre to post exercise.[30] Another study looking at collegiate football players demonstrated 9 out of 10 athletes consistently lost body weight from not adequately replacing fluid loses.[3] USG measurements in athletes were consistently higher after practice, with loses of up to 1.5-2% body weight during practice.[3] These athletes were unable to properly replace fluid losses and regularly began practice in a hypohydrated state.[3]

Other adult athletes that have been regularly studied regarding hypohydration include marines and runners. Of Marines who were involved in foot patrol over a 24-hour period, 90% were dehydrated and become further dehydrated as they exerted themselves.[31] This could be particularly problematic when exposed to extreme environments such as hot temperatures and prolonged exposure to direct sunlight. In a study of runners looking at hydration status, hypohydration resulted in slower finishing times, higher core body temperatures, increased

environmental symptoms, slower recovery, increased perception of warmth and exertion, and increased perception of thirst.[4] A study looking at the injuries obtained by runners during the Baltimore Marathon demonstrated that dehydration was the main complaint in 32% of all injuries.[32] Another study looking at Ironman triathletes discovered an average body weight loss of approximately 3%.[33]

Measures of Hydration Status

Blood

Measures of hydration status taken from the blood include plasma osmolality and plasma volume shifts. Plasma osmolality is performed with either a freezing point or vapor pressure depression osmometer.[34] Plasma osmolality readings must be done immediately and requires a great deal of processing time after the sample is taken[34], making it impractical for use in a clinical setting. However, plasma osmolality is the most used hematological index of hydration status, with many considering it the only valid means of assessing hydration.[34]

Plasma volume shifts can also be used to measure hydration status. Plasma volume is the liquid portion of the blood.[34] In dehydrated individuals, extracellular fluids migrate out of the blood circulation, decreasing plasma volume and hindering the body's thermoregulation ability.[34] Due to a decreased amount of plasma, proteins in the cell become much more concentrated.[35] On average, in dehydrated individuals, plasma volume decreases 9.6%.[35] Plasma volume is assessed by drawing venous blood from the antecubital vein and measuring hematocrit and hemoglobin levels at two separate times, which are used to calculate the shift in plasma volume.[34] Osmolality affects the brain by stimulating osmoreceptors when fluid levels get low, creating a thirst response in the brain.[36]

Urine

Urine specific gravity (USG) is considered a valid measure of hydration status and is the most practical and cost-efficient.[2,7,28] USG is the ratio of densities between urine and water.[16] Assessing the density of urine provides an indication of hydration status because urine that is denser is more concentrated, thus containing less fluid content.[34] Normal USG ranges between 1.00 and 1.030, with minimal dehydration from 1.020-1.024, significant dehydration from 1.025-1.029, and serious dehydration any value greater than 1.030.[7,27] Devices used for measuring USG include refractometer and reagent strips. Refractometry is the preferred method in the field, as it is a more sensitive indicator of hydration status.[7] Refractometry estimates USG by determining the urine's refractive index, the ratio of velocity of light in the air to the velocity of light in urine.[37] USG can be measured quickly through the use of a refractometer by placing a drop of urine specimen on the stage and pointing it towards a light source.[34] Urine reagent strips, on the contrary, often have unpredictable results and are less accurate than refractometry.[7,37] Reagent strips can be easily misused due to the wide variety of manufacturers, each containing a specific set of instructions for immersion timing and result reading.[7] Reagent strips contain a polymer of methyl vinyl ether/maleic anhydride that are attracted to salt-containing solutions like urine, releasing protons that change the color by reacting with the pH indicator present on the strip.[37] Reagent strips provide the user with much greater convenience, low cost, and disposability but are not as accurate as refractometry.[7,37]

Urine color is another indicator of hydration status that is often used, especially in athletic settings. It utilizes a urine color chart, which consists of a scale with colors ranging from pale (1) to brownish green (8).[34] Upon collection of a urine sample, the sample is held up to the color chart and matched with a corresponding color and number. Urine colors numbered 1, 2, or

3 indicate a hydrated state while colors numbered 4 through 8 indicate some degree of dehydration. Urine color has been proven to be an effective tool in educating athletes on their hydration status.[7] Although good for education of athletes, urine color is less effective than urine reagent strips in assessing hydration status of athletes.[7,34]

Urine osmolality is considered the standard for measuring total solute concentration and is, therefore, the best method for estimating the kidney's concentrating ability.[7] Osmolality is determined through the use of an osmometer, which measures the total solute concentration and compares the specimen to the freezing point of water or the amount of osmoles of solute per kilogram of solution.[7,16] This method is unlikely to be used for athletic training clinical or field setting due to high costs and the technical knowledge required to operate.[7]

Other methods of assessing hydration status from urine include 24-hour urine volume and urine conductivity. 24-hour urine volume can be an easy method for an individual to assess hydration status. Healthy values for women are around 1.13 L, with a minimum of 0.29 L per day.[34] Healthy values for men are 1.36L, with a minimum of 0.48L per day.[34] In order to avoid falling two standard deviations below the mean, women and men should produce at least 0.29 and 0.48 L/day respectively.[34] Elderly and children produce proportionately smaller quantities daily.[34] Urine conductivity is dependent upon the concentration of salts.[38] Urine conductivity has been shown to produce valid results and is a portable, easy to use tool for providing instant feedback to athletes and coaches.[34,39]

Thirst

In addition to looking at urine color, the thirst scale provides another subjective means to assessing hydration status. The onset of thirst typically develops when total body weight losses reach 1-2% body weight.[34] The thirst scale consists of an athlete's perception of thirst measured

with a numerical rating scale. The scale ranges from 1, not at all thirst, to 9, very, very thirst.[34] Thirst perception that falls between the ranges of 3-5 is considered mildly dehydrated. The thirst scale works on the notion that dehydrated individuals show an increased thirst response.[40] Water is an essential part of normal cellular function in the body and is constantly lost from the kidneys, lungs, and skin.[36] In order for these systems to function normally, fluids must be replaced. When fluid levels are low, osmoreceptors are stimulating and cause a thirst response in the brain.[36] The perception of thirst can be altered by many things, including palatability of the fluid, time allowed for fluid consumption, gastric distention, gender, age, and heat acclimatization.[34]

Changes in Body Mass

Weight lost during exercise generally corresponds with water loss due to increased perspiration, making changes in body mass an effective tool for detecting dehydration.[16] Measuring body mass changes is a simple, non-invasive method for testing hydration of athletes before and after practice.[34] Body weight changes are determined by measuring body weight before and after practice and calculating the percentage of body weight lost. Body weight losses of 1-2% can compromise physiological functioning and negatively affect performance.[6] When body weight losses exceed 3%, physiological functioning continues to deteriorate and the risk of developing EHI increases.[6] Although it is effective in the short term, this method of measuring hydration status cannot be used over long periods of time due to increases and decreases in adipose tissue.[34] In addition, one study looking at the daily variability in body mass measurements found that 3 consecutive measurements of body weight are required in order to provide an accurate assessment of the daily variability.[41]

Specimen Methodology

A literature review was done of current manuals and books that contain procedure guidelines for the handling and/or collection of urine specimens. It is typically preferred to utilize the first void of the day, as the specimen are usually more concentrated.[10] In addition samples should be analyzed as soon as possible after they are collected, specifically within two hours, as bacteria may begin to grow.[10,14] It is important to note that none of the manuals cited in this review of literature on methodology of urine analysis cited references within their documents. In addition, there was no mention of the use of agitation in any procedure manual or any other technique once sedimentation has formed.

Institutional Guidelines

The NATA currently recommends USG as the primary measurement of hydration status with the use of a clinical refractometer in conjunction with urine color and changes in body mass.[6] The NATA also encourages the education of athletes on the risk of hypohydration and an individualized fluid replacement plan.[6]

The ACSM recommends daily body mass changes as an Evidence Category A.[8] Simple urine and body weight measurements, along with USG are recommended as an Evidence Category B.[8] The ACSM also encourages individuals to begin activity in a euhydrated state, and drinking fluids during exercise to prevent excessive dehydration.[8]

The military currently recommends the use of body weight changes as the primary method of measuring hydration status.[9] Individuals should be encouraged to consume the proper amount of fluid to support their work rate.[9] Specifying upper level limits for hourly and daily water intake is also recommended to ensure proper fluid replacement.[9]

USA Track and Field currently recommends the use of a self-testing program for optimal hydration, which involves athletes calculating their sweat rates to ensure rehydration needs are being met.[42] This advisory encourages all athletes to calculate approximate sweat rates for a range of environmental conditions, practice, and competitions.[42]

The National Collegiate Athletic Association (NCAA) Wrestling Weight Management Program recommends the use of USG as the most practical, cost-effective measure of hydration.[43] NCAA wrestlers must be in a hydrated state, specific gravity less than or equal to 1.020, in order to record a baseline weigh-in.[43]

Summary

Athletes and active individuals are affected by hypohydration before, during, and after physical activity. With many severe physiologic and performance implications caused by hypohydration, it is important to determine the best means possible of evaluating hydration status. Currently, urinary specimen collection procedures are out of date and lack scientific evidence to support their recommendations. Unfortunately for athletic trainers, health care providers, and researchers, immediate examination of samples is not always plausible. In these instances, it would be ideal to know if some form of agitation should be used on a sample in order to ensure the most valid measurement of hydration status. There is currently no information in the literature or in procedure manuals for urinary specimen collection regarding the use of agitation.[10,44] Therefore, the purpose of this study is to determine if agitation of urine samples is comparable to the criterion measure.

CHAPTER 3

METHODS

Research Design

This study is a descriptive diagnostic validity test design. The criterion measure is urine osmolality as measured by an osmometer within 2 hours of sample collection. Concurrent validity will be assessed for each agitation type compared to the criterion sample. Three types of agitation tested will include "hand shaken," "vortex," and "no shake."

Participants

Approximately 500 samples from males and females between the ages of 18-60 years will be recruited from the Indiana State University community to participate in the study. Participants will be informed of risks and benefits of participation in the study, and will complete an informed consent approved by the Institutional Review Board prior to participation in the study. There will be no other inclusion or exclusion criteria.

Instrumentation

Urine Osmolality

Urine osmolality will be measured by the use of an osmometer (Model 3320, Advanced Instruments Inc., Norwood, MA). The osmometer uses the freezing point depression technique to measure the total solute concentration by comparing the specimen to the freezing point of water (1.86^{o}C).[7,37] Osmolality is considered the gold standard for determining urine concentration and kidney function estimation.[7] The osmometer will be calibrated at the

beginning of each data collection session, as instructed by the manufacturer's instructions. Duplicate measurements will be used. However, when the two samples are greater than 1 percent apart, a third measurement will be done, and the median results will be used.[45]

The following testing procedures will be followed for all osmolality measurements. First, a tip will be inserted on the sampler and a 20-μL sample free of large voids or bubbles will be extracted. The sampler tip will then be wiped, removing any remaining droplets or protruding fluid from the tip. Next, the sample will be inserted into the sample port so that it is resting in the operating cradle and will be pushed into the unit until it stops, starting the test. Each sample will take approximately one minute and the results will be displayed in the format "Osmolality xxx mOsm".

Vortex

Thermo Scientific Vortex Maxi Mixer (Model M16715) will be used to perform agitation of the vortex labeled specimen. The specimen will be placed on the machine and agitation will be applied for ten seconds at 3,000 revolutions per minute.[46]

Procedures

Each participant will complete a health questionnaire asking them to self-report age, gender, height, weight, time of void, menstruation, physical activity in the past 24 hours, and presence of any of the following conditions: chronic kidney disease, diabetes, recurrent urinary tract infection, and supplement usage. Participants will be provided with a clean specimen cup and asked to use the restroom in order to provide a sample. Large samples will be encouraged as samples will be immediately split into three cups and labeled according to participant number and agitation method (hand shaken [HS], vortex [Vtx], or no shake [NS]). All samples will be

stored in the biochemical research laboratory in a thermoneutral environment. New osmolality tips will be used for each measurement to reduce the risk of contamination.

Within two hours of collection, each sample will be analyzed using the osmometer to determine hydration status, serving as the criterion measure (control). After forty-eight hours, each sample will be agitated in the appropriate manner and hydration status will be determined by the use of an osmometer. Each HS sample will be agitated ten times in an hourglass fashion, from right side up to up side down. Vtx samples will be placed on the vortex mixer for 10 seconds at 3,000 RPM. NS samples will not be disturbed. Participant recruitment and data collection will occur in approximately bi-monthly installments with 15 to 20 samples being processed at a time.

Statistical Analysis

Following data collection, descriptive statistics will be calculated for all agitation types and a one-way analysis of variance (ANOVA) will compare the two methods of agitation against the criterion control. The control sample and criterion for validity will be the original NS specimen analyzed on the first day. Significance will be set at $p<0.05$ a-priori. In order to effectively achieve the necessary power ($1-\beta=0.95$) and effect ($f=0.25$ [medium]) for this investigation, a minimum of 305 samples are needed.

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CHAPTER 4

MANUSCRIPT

Effects of Agitation on Measurements of Hydration Status

Introduction

Hypohydration can have significant implications on normal physiological functions of the body.[1,2] The most substantial effects can occur on the thermoregulatory and cardiovascular systems, diminishing the ability of the body to dissipate heat efficiently.[3,4] This decreased ability to dissipate heat can increase susceptibility to life-threatening conditions such as exertional heat stroke.[3] In addition, current research suggests hypohydration has the potential to increase fatigue and decrease race times, ultimately compromising performance.[1,2]

Several methods for determining hydration status have been discussed in literature including plasma and urine osmolality, urine specific gravity, urine color, thirst scale, 24-hour urine volume, and monitoring changes in body mass.[5] Urine osmolality is suggested to be the most accurate indicator of total solute concentration, but is not always practical in a clinical setting due to high costs and technical knowledge required for operation.[5,6] Although urine specific gravity is not the gold standard, current literature suggests a clinical refractometer is valid and more practical for determining hydration status in clinical settings.[5]

Much of the current literature is out of date and does not provide sufficient evidence to support recommendations on proper procedures for urine specimen collection and storage.[7-10] The limited research available has primarily included instrumentation recommendations, but has

not yet looked at the impact of agitation on a sample after being stored for a period of time. Clinicians often obtain urine specimen samples as preventative measures to assess athlete hydration status for weight checks and monitoring body mass losses. Unfortunately, immediate examination of urine samples is not always possible. Sedimentation may develop as the urine samples sits and current literature does not address this visible sedimentation or the procedures clinicians should follow to ensure an accurate hydration assessment after a sample sits. The purpose of this study was to determine if agitation of urine samples is comparable to the criterion measure, urine osmolality measured within two hours of collection. The secondary purpose of this study was to determine if analysis of a urine specimen is necessary within two hours of collection.

Methods

We used a descriptive diagnostic validity test design to investigate the effects agitation of urine samples has on the measurement of hydration status. We compared hydration status across three levels of agitation: vortex mixed (Vtx), hand shaken (HS), and no agitation (NS). The dependent variable was hydration status, determined by a freezing point depression osmometer.

Participants

We recruited seventy-five (41 males, 34 females) healthy individuals (mean age=22±5years; mean self-reported height=172±23cm and mass=77±17kg) to participate in this study, providing one or more samples (81 total samples). Each participant completed a short health questionnaire to self-report demographics and rule out any exclusion criteria such as kidney disease, diabetes, etc. The university's Institutional Review Board approved this study and all participants completed the written informed consent process.

Instrumentation

Urine Osmolality

We measured urine osmolality using an osmometer (Model 3320, Advanced Instruments Inc., Norwood, MA). This osmometer utilizes the freezing point depression technique to measure the total solute concentration by comparing the specimen to the freezing point of water (1.86^{o}C). [5,11] We calibrated the osmometer prior to each data collection session, as instructed by the manufacturer. We analyzed each sample in duplicate, but in the event two samples were greater than 5mOsm apart, we analyzed the sample in triplicate and we used the mean of the three results for statistical analysis.

To analyze each sample, we inserted the tip of the sampler to extract a 20-µL sample, free of large voids or bubbles. We wiped the sampler tip, removing any remaining droplets or protruding fluid from the tip. We inserted the sampler into the sample port until resting in the operating cradle and then pushed into the unit, beginning the test. After approximately one minute, the osmometer displayed each result in the format "Osmolality xxx mOsm."

Vortex

We used the Thermo Scientific Vortex Maxi Mixer (Model M16715) to perform agitation of vortex labeled samples. We placed each Vtx sample on the vortex machine for 10 seconds at 3,000 revolutions per minute.

Procedures

We provided each participant with a clean specimen cup and directed them to use the restroom to provide a urine sample. We encouraged participants to provide large samples as we split each into three cups labeled according to participant number and agitation method. Within two hours of collection, we analyzed each sample using the osmometer to determine hydration

status, serving as the control and criterion for validity. Samples were stored in the biochemical research laboratory in a thermoneutral environment. After forty-eight hours, we agitated each sample in the appropriate manner to determine hydration status with the osmometer. To hand shake samples, we tipped the sample 10 times in an hourglass fashion, from right side up to up side down. We performed agitation on Vtx samples by placing the sample on a vortex mixer for 10 seconds. NS samples were not disturbed.

Statistical Analysis

We used a one-way analysis of variance (ANOVA) to compare the three methods of agitation against the criterion control. The control sample and criterion for validity was the original specimen analyzed within two hours of collection. We set significance at $p<0.05$ a-priori.

Results

We identified no significant differences ($F_{3,316} = 0.00027$, $p = 0.99$, $1-\beta=1.00$) between the NS (mean=724±262), HS (mean=723±263) and Vtx (mean=724±263) methods when compared to the criterion control (mean=723±262). Mean osmolality measurements across all agitation types are expressed in Figure 1.

Discussion

While instrumentation has been the focus of previous studies, this study was the first to investigate the effects of agitation on urine samples. The purpose of this study was to determine if agitation of urine samples is comparable to the criterion measure, urine osmolality measured within two hours of collection. We observed no differences between agitation methods and the criterion.

Urine collection procedure manuals have suggested urine samples should be analyzed within two hours of collection.[7,12] In contrast, the results of this study demonstrate no differences between samples receiving no agitation after forty-eight hours and the control measured within two hours of collection. From a clinical perspective, these findings suggest immediate analysis of urine samples is not necessary to obtain a valid measurement of hydration status. Instead, a sample can be stored for up to 48 hours without effecting hydration status measurements. Additionally, we observed no differences between agitation methods. A possible explanation for the lack of differences between the three agitation methods and the control is the time-frame in which samples were stored. We did not observe visible sedimentation after 48 hours in most samples. While analysis within 48 hours seems practical for most clinicians, additional storage time may have resulted in further sedimentation that could have effected the hydration measurement.

We used an osmometer to assess hydration status in the present study. Previous literature has investigated different instrument types available for hydration status measurement. Research suggests urine specific gravity is a valid method for assessing hydration that is both practical for clinicians and cost-efficient.[5,13,14] Urine osmolality is considered the standard for measuring total solute concentration despite being less practical than other forms of hydration assessmet.[5,15] Changes in body mass, thirst scale, and 24-hour urine volume have also been discussed in the literature. As suggested by literature, we used an osmomter to ensure a valid measure of hydration.

Clinicians today turn to manuals for proper urine collection and handling procedures. These manuals are out of date and lack any research to support their recommendations. Manuals suggest clinicians should utilize the first void of the day, as specimens are more concentrated.[7]

We did not require the first void of the day from participants due to timing and availability of collection times both in this study and the clinical setting. Practice times change daily and are often not early in the morning when health care providers can obtain first voids. Clinicians are encouraged to analyze samples within two hours of collection in order to avoid excessive bacteria growth.[7,12] We used similar methods in this study, ensuring all samples were analyzed within two hours of collection. Current urine collection manuals do not mention the use of agitation or any other technique clinicians should utilize once sedimentation has developed. We investigated agitation via hand shaking and vortex mixer, methods we believed to be practical for both laboratory analysis as well as clinical settings where equipment may not be available.

To our knowledge, there have been no previous studies investigating the effects of agitation on measurements of hydration status. The results of the present study indicate agitation does not have an effect on measurements of hydration status. From a clinical perspective, this is important because previous literature provides clinicians with no guidance on how to handle sedimentation. Agitation of the urine specimen is not necessary for clinicians who are unable to immediately analyze measurements of hydration.

Conclusion

The purpose of this study was to determine if agitation of urine samples is comparable to the criterion measure, urine osmolality measured within two hours of collection. The findings of this study demonstrated no differences in hydration status measurements between the three agitation methods and the criterion control. For practitioners who are unable to immediately measure the hydration status of urine samples, agitation of the urine specimen is not necessary when no visible sedimentation is observed in order to obtain a valid measure of hydration status

using an osmometer. Additionally, immediate analysis within two hours of collection is not necessary.

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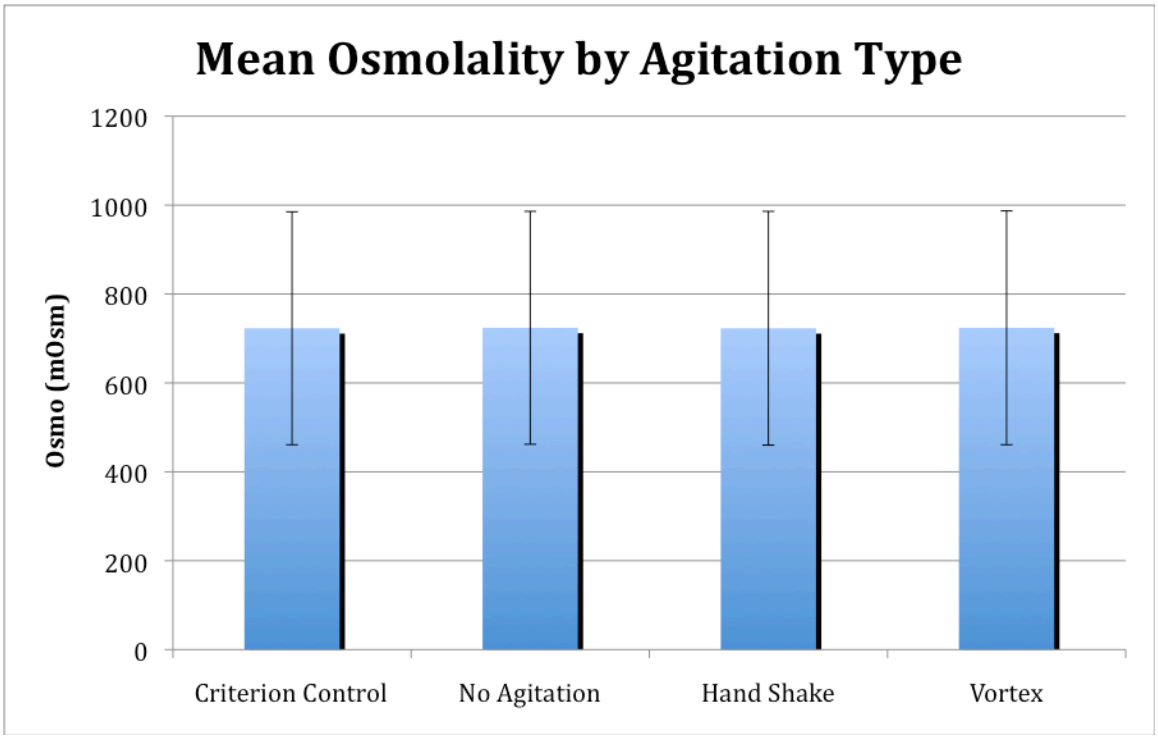
Figure 1. Mean osmolality measurement by agitation type.



($F_{3,316}$ = 0.00027, p =0.99, 1-β=1.00)

APPENDIX A: STUDY PARAMETERS

Operational Definitions

Euhydration: Euhydration is the steady-state condition of normal body water

Hypohydration: Hypohydration is the steady-state condition of decreased body water.

Urine Osmolality: Osmolality is determined through the use of an osmometer, which measures the total solute concentration and compares the specimen to the freezing point of water or the amount of osmoles of solute per kilogram of solution.

Urine Specific Gravity (USG): USG is the ratio of densities between urine and water. Devices used for measuring USG include refractometer and reagent strips.

Agitation: The action of stirring or disrupting an item or substance.

Assumptions

- Health questionnaires were self-reported, so we assume participants provided accurate information.
- Participants will provide their own urine sample.
- We assumed a rate and frequency of 10 seconds at 3,000 RPM on the vortex machine was adequate time for agitation.
- We assumed all hand shaken agitation was performed in the same manner.

Delimitations

- The results of this study are limited to individuals between the ages of 18 and 60.
- Agitation methods of hand shaken and vortex.

Limitations

- We were unable to standardize hand shaken procedures.
- Since we did not observe visible sedimentation, the results of this study are limited to urine specimen without sedimentation.
- We were unable to obtain specific information on the supplements or vitamins participants were consuming.

APPENDIX B: RELEVANT STUDY FORMS

Participant Health Questionnaire

Appendix C. Health Questionnaire

Participant #

Health Questionnaire

Study Title: Validation of Hydration Status Measurement Methodology: A Five Part Investigation

Mass: _____ Gender: M

Height:_____in lbs Age:_____yrs or F

Please answer the following questions to the best of your knowledge:

Questions		
1. Is this your first time urinating today? If not please list how many times you have urinated today _____	*Yes*	*No*
2. Have you been diagnosed with diabetes?	*Yes*	*No*
2. Do you have a history of chronic urinary tract infections?	*Yes*	*No*
3. Have been diagnosed with kidney disease?	*Yes*	*No*
4. Are currently taking any supplements or vitamins?	*Yes*	*No*
5. Approximately how much have you exercised in the past 24 hours?	_____*hours*	
6. *Females only*- Are you currently menstruating?	*Yes*	*No*

* This information is confidential and will be used for descriptive purposes only. This information will not exclude you from the study or lottery.

Data Collection Form

Indiana State University
More. From day one.

Validation of Urine Hydration Status Measurement Methodology: A Five Part Investigation

	Participant #												
<2hrs(control)	DR	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	CR	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Hyg												
	Osmo												
Day 2	Control Osmo												
	Fridge Osmo												
Day 3	Control Osmo												
	Fridge Osmo												
	Freezer Osmo												
Sedimentation	HS												
	Vtx												
	Day #												

Institutional Review Board Informed Consent Form

CONSENT TO PARTICIPATE IN RESEARCH

Validation of Urine Hydration Status Measurement Methodology: A Five Part Investigation

You are asked to participate in a research study conducted by Dr. Susan Yeargin, Dr. Lindsey Eberman, Heather Mata, Heather Adams, and Andrew Niemann, members of the Department of Applied Medicine and Rehabilitation at Indiana State University. Your participation in this study is voluntary, so at any time, you can discontinue without any consequences. Please read the information below and ask questions about anything you do not understand, before deciding whether or not to participate.

• PURPOSE OF THE STUDY

Urine is commonly used to determine a person's hydration status by researchers and health care providers. Current research is unclear about the best ways to evaluate a urine sample. The goal of this study is to determine whether factors like time, shaking, temperature, number of times urinating, and measurement type change the results of a urine sample.

• PROCEDURES

If you volunteer to participate in this study, you will be asked to do the following things:

- Complete a health questionnaire
- You will be given a clean urine specimen cup
- Go to the restroom with the cup, making sure to lock the door behind you
- Provide as much urine as possible in the sample cup
- Wash your hands and leave the urine sample in the restroom for the researchers to analyze later

• POTENTIAL RISKS AND DISCOMFORTS

We expect the risks for this study will be minor. If your discomforts become a problem, you may choose to discontinue your participation at any time. Possible risks that may be experienced include you becoming socially uncomfortable due to the process of urine collection and transportation of urine. Allowing you to leave your sample in the bathroom will help minimize this risk.

• POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY

It is unlikely you will directly benefit from participation in this study. However, this research will help increase the awareness and education on the importance of hydration in addition to generating standardized procedures, for both clinical and research purposes, for assessing hydration status.

• PAYMENT FOR PARTICIPATION

If you choose to participate, you can also choose to enter a lottery for a $20 Wal-Mart gift card. Ten gift cards will be distributed at the conclusion of the study based on a random drawing of email addresses. Please indicate below whether you would like to be included in the lottery. If you choose not to enter the lottery, you can still provide a urine sample for analysis. You can also choose to provide more than one sample, but your name will only be entered into the drawing once.

Please note: Foreign nationals on visas other than F-1 or J-1 may not be eligible to receive payment for participation in this study.

Place a check in the box to indicate your choice:

☐ I DO want to enter my name in the lottery. ☐ I DO NOT want to enter into the lottery.
Email address: _____

- **CONFIDENTIALITY**

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission or as required by law. Confidentiality will be maintained by means of assigning you a subject number. The only location where your subject number and name will be together will be in a file on the primary investigator's password protected computer. Only the investigators will have access to this file. This consent form (which only has your name) and the health questionnaire (which only has your subject number) will be stored in a locked cabinet in a locked office in the Applied Medicine Research Laboratory. Only the primary investigators will have access to these files. If you choose to discontinue participation at any time, all forms related to your participation will be immediately destroyed.

- **PARTICIPATION AND WITHDRAWAL**

You can choose whether or not to be in this study. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind or loss of benefits to which you are otherwise entitled. You may also refuse to answer any questions you do not want to answer. There is no penalty if you withdraw from the study and you will not lose any benefits to which you are otherwise entitled.

- **IDENTIFICATION OF INVESTIGATORS**

If you have any questions or concerns about this research, please contact

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- **RIGHTS OF RESEARCH SUBJECTS**

If you have any questions about your rights as a research subject, you may contact the Indiana State University Institutional Review Board (IRB) by mail at Indiana State University, Office of Sponsored

Programs, Terre Haute, IN 47809, by phone at (812) 237-8217, or e-mail the IRB at irb@indstate.edu. You will be given the opportunity to discuss any questions about your rights as a research subject with a member of the IRB. The IRB is an independent committee composed of members of the University community, as well as lay members of the community not connected with ISU. The IRB has reviewed and approved this study.

I understand the procedures described above. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____
Printed Name of Subject

_____ _____
Signature of Subject Date

Leave this amount of space for IRB approval stamp (unless you plan to include the approval information in the text of the ICD)

APPENDIX C: RAW DATA

	<2hrs(control)				Day 3 (48 Hours)											
Sample #	Control Osmo			Mean	NS			Mean	HS			Mean	Vtx			Mean
2	247	246		246.5	242	243		242.5	243	243		243	242	242		242
4	569	570		569.5	569	571		570	572	570		571	571	570		570.5
6	878	883		880.5	895	890		892.5	878	883		880.5	883	881		882
8	990	983	981	984.7	987	984		985.5	983	988		985.5	986	979	984	983
10	615	617		616	609	615	612	612	613	613		613	615	613		614
12	662	668	665	665	668	664		666	661	663		662	667	661	662	663.3
14	1025	1013	1017	1018	1013	1013		1013	1019	1012	1018	1016	1024	1018	1008	1017
16	895	888		891.5	887	882		884.5	890	894		892	896	892		894
18	841	836		838.5	844	839		841.5	842	840		841	844	847		845.5
20	521	522		521.5	521	522		521.5	516	517		516.5	516	520		518
22	1238	1206	1216	1220	1227	1212	1212	1217								
24	939	930	933	934	939	936		937.5	940	937		938.5	948	939	939	942
26	370	370		370	378	375		376.5	373	373		373	373	371		372
28	811	812		811.5	815	808	806	809.7	816	816		816	815	809	805	809.7
30	239	239		239	238	239		238.5	238	239		238.5	239	238		238.5
32	877	878		877.5	875	878		876.5	876	877		876.5	881	881		881
34	141	143		142	143	141		142	141	141		141	144	142		143
36	550	543	545	546	542	543		542.5	541	549	540	543.3	540	541		540.5
38	570	572		571	574	575		574.5								
40	634	634		634	637	639		638	635	633		634	632	630		631
42	874	874		874	871	874		872.5	869	873		871	873	870		871.5
44	1012	1009		1011	1020	1017		1019								
46	950	953		951.5	952	957		954.5	954	952		953	946	954	950	950
48	737	746	747	743.3	754	743	739	745.3	752	731	744	742.3	740	745		742.5
50	900	904		902	902	902		902	901	902		901.5	905	907		906
52	1038	1042		1040	1036	1037		1037	1039	1035		1037	1039	1032	1038	1036
54	730	727		728.5	721	724		722.5	726	719	721	722	722	723		722.5
56	926	923		924.5	921	922		921.5	935	922	924	927	922	925		923.5
58	1103	1095	1098	1099	1091	1101	1093	1095	1101	1093	1100	1098	1100	1100		1100
60	799	804		801.5	816	821		818.5	817	815		816	824	824		824
62	984	989		986.5	989	997	991	992.3	996	990	986	990.7	993	992		992.5
64	1041	1046		1044	1035	1036		1036	1038	1043		1041	1034	1038		1036
66	975	964	968	969	971	971		971	971	976		973.5	967	972		969.5
68	1123	1126		1125	1125	1128		1127	1125	1128		1127	1132	1128		1130
70	150	151		150.5	154	155		154.5	155	151		153	151	151		151
72	839	843		841	854	854		854	850	845		847.5	850	849		849.5
74	787	788		787.5	793	790		791.5	786	789		787.5	793	785	791	789.7
76	668	677	673	672.7	678	676		677								
78	721	722		721.5	734	737		735.5	728	729		728.5	724	729		726.5
80	904	912	899	905	903	906		904.5	903	898		900.5	902	898		900
82	480	478		479	478	478		478	486	477	476	479.7	479	476		477.5
84	476	476		476	477	474		475.5	477	474		475.5	480	477		478.5
86	947	949		948	934	941	930	935	945	939	941	941.7	943	948		945.5
88	828	826		827	829	832		830.5								
90	452	454		453	457	451	453	453.7	454	451		452.5	455	452		453.5
92	800	805		802.5	803	806		804.5	804	797	804	801.7	802	801		801.5
94	370	371		370.5	365	366		365.5	367	369		368	369	366		367.5
96	500	499		499.5	495	497		496	499	496		497.5	497	498		497.5
98	494	494		494	496	496		496	493	495		494	496	495		495.5
100	816	815		815.5	797	900		848.5	807	809		808	806	809		807.5
102	1069	1069		1069	1064	1065		1065	1073	1075		1074	1071	1074		1073
104	874	877		875.5	872	877		874.5	872	875		873.5	886	874	879	879.7
106	371	372		371.5	371	367		369	371	369		370	371	370		370.5
108	896	897		896.5	892	888		890	888	888		888	884	881		882.5
110	459	459		459	455	455		455								
112	916	907	907	910	918	914		916	913	914		913.5	914	910		912
114	771	778	778	775.7	771	776		773.5	774	770		772	772	770		771
116	926	927		926.5	920	920		920	923	917	921	920.3	919	928	922	923
118	464	460		462	455	455		455	455	453		454	457	454		455.5
120	1050	1055		1053	1055	1051		1053	1047	1052		1050	1058	1056		1057
122	271	270		270.5	265	265		265	271	269		270	272	270		271
126	906	902		904	916	913		914.5	910	916	912	912.7	908	911		909.5

APPENDIX D: STATISTICAL ANALYSIS

Descriptives

Osmolality

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Between-Component Variance
						Lower Bound	Upper Bound			
Control		80	723.41	262.219	29.317	665.06	781.76	142	1125	
No Agitation		80	723.47	262.390	29.336	665.08	781.86	142	1127	
Hand Shaken Agitation		80	722.73	262.516	29.350	664.31	781.15	141	1127	
Vortex Agitation		80	723.90	263.369	29.446	665.29	782.51	143	1130	
Total		320	723.38	261.386	14.612	694.63	752.13	141	1130	
Model	Fixed Effects			262.624	14.681	694.49	752.26			
	Random Effects				14.681[a]	676.66[a]	770.10[a]			-861.907

a. Warning: Between-component variance is negative. It was replaced by 0.0 in computing this random effects measure.

Test of Homogeneity of Variances

Osmolality

Levene Statistic	df1	df2	Sig.
.001	3	316	1.000

ANOVA

Osmolality

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.251	3	18.750	.000	1.000
Within Groups	2.179E7	316	68971.303		
Total	2.179E7	319			

Post Hoc Tests

Multiple Comparisons

Osmolality

Bonferroni

(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	No Agitation	-.060	41.524	1.000	-110.31	110.19
	Hand Shaken Agitation	.681	41.524	1.000	-109.57	110.93
	Vortex Agitation	-.490	41.524	1.000	-110.74	109.76
No Agitation	Control	.060	41.524	1.000	-110.19	110.31
	Hand Shaken Agitation	.742	41.524	1.000	-109.50	110.99
	Vortex Agitation	-.429	41.524	1.000	-110.68	109.82
Hand Shaken Agitation	Control	-.681	41.524	1.000	-110.93	109.57
	No Agitation	-.742	41.524	1.000	-110.99	109.50
	Vortex Agitation	-1.171	41.524	1.000	-111.42	109.08
Vortex Agitation	Control	.490	41.524	1.000	-109.76	110.74
	No Agitation	.429	41.524	1.000	-109.82	110.68
	Hand Shaken Agitation	1.171	41.524	1.000	-109.08	111.42

APPENDIX E: RECOMMENDATIONS

Methodology

We did not observe visible sedimentation of samples after 48 hours of storage in a thermoneutral environment. In an attempt to observe sedimentation, we should have increased storage time. Additionally, we could have stored samples in a thermal environment to encourage bacterial growth and increase the likelihood of sedimentation formation.

Future Research

While the findings of this study have valuable implications for the clinical practice of urine collection and handling, additional research is necessary. Future research should incorporate various urine storage temperatures, which may directly influence the formation of sedimentation. Additionally, we should expand the age of participants to include youths.