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Utility of Urine Samples for Biomarker Collection in Pediatric Studies

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Conflict of Interest
The authors declare that there is no conflict of interest.

Abstract

Background: Biobanks are critical tools for advancing scientific knowledge. Barriers to collecting biological samples exist for all clinical populations but remain especially understudied in pediatric patients. Literature focused on the ability to biobank blood and urine specimens in children is underexplored.

Objective: This retrospective study is an exploratory, secondary analysis of a longitudinal parent study to evaluate the availability of serial blood and urine samples for biobanking, when collected as part of standard of care.

Methods: Children admitted to a university-affiliated hospital for traumatic brain injury were enrolled into a parent study ($N = 60$), which collected serial biologic samples. The analysis included 37 children, of which 75.7% ($n = 28$) were Caucasian, the majority were male 59.5% ($n = 22$), and 94.6% ($n = 35$) were diagnosed with mild traumatic brain injury. Participant injury characteristics and clinical data were abstracted from electronic health records, and statistics were generated to explore differences in biospecimen availability by type.

Results: On day 1, urine samples were available 2.46 times more often than blood samples. By day 5, 13.5% of participants were still providing urine when compared to only 2.7% for blood. Over the five-day period 18.9% switched from providing both blood and urine samples to providing urine only.

Conclusions: These findings demonstrate that urine is more readily available than blood as a biospecimen when collected in children for biomarker analysis. Future studies should evaluate the utility of urine biomarkers for diagnostic and prognostic purposes.

Keywords: *biospecimen; biomarker; urine; blood; pediatrics*

Background

Biological markers, better known as "biomarkers", are proteins or other biomolecules that can be objectively measured. Sources of biomarkers include blood, urine, cerebrospinal fluid (CSF), saliva, hair, and other tissues. Biomarkers are often evaluated as an indicator of normal biologic functions, pathogenic processes, or pharmacologic responses; these applications have become common in clinical research and practice (Strimbu & Tavel, 2010). Medical applications of well-vetted biomarkers are numerous and include diagnosis, prognosis, and monitoring of therapeutic response, including the use of Glial fibrillary acidic protein (GFAP), Interleukin 6, (IL-6), Tumor Necrosis Factor alpha (TNFa), Ubiquitin C-terminal hydrolase L1 (UCHL1), and S100 calcium-binding protein B (S100B) in the diagnosis of traumatic brain injury (TBI) (Chaban et al., 2020; Dadas et al., 2018; Kulbe & Geddes, 2016; Vos, et al., 2010; Yang et al., 2013). Urinary biomarkers are used for kidney disease and beyond, such as in the study of Alzheimer' disease, Parkinson's disease, and other neurologically-based diseases (An & Gao, 2015; Kurbatova et al., 2020; Lisowska-Myjak, 2010; Seol, Kim, & Son, 2020). The majority of FDA-approved biomarkers are for use in adults, while pediatric biomarker research lags; leaving an important gap because adult biomarker data cannot be presumed applicable to pediatric patients. An example of this gap is found by Oris et al. (2018) in that the child's age is essential in the interpretation of S100 calcium-binding protein B concentrations because protein values vary physiologically during the first two years of life. Promising biomarkers in adult populations should be vetted in children to confirm their utility. This finding necessitates additional pediatric research studies that include biobanking of biologic samples for future biomarker analysis.

There are many practical barriers to collecting biospecimens in children, such as the desire to avoid painful blood draws and limitations in amount of blood that can be drawn; these challenges have been extensively discussed but have not been empirically studied (Davit et al., 2011; Duff, 2003; Howie, 2011). The quest for clinically predictive biomarkers of pediatric disease and/or injury must include considerations for the practicality of sample collection in the context of clinical care, as these can impact the ability to effectively conduct biomarker research and ultimately clinical translation efforts.

Despite the known impact of clinical realities on biobanking, no study has addressed longitudinal collection of biospecimens when it was feasible to dovetail research sample collection as part of the standard of care. This line of inquiry is relevant to promoting collection of future research specimens, while avoiding the need for additional invasive and painful sample collection procedures. The purpose of this exploratory pilot study was to leverage data from a longitudinal biobanking parent study which dovetailed collection of blood and urine with standard of care activities to assess differences in availability of these biospecimens in children hospitalized for TBI.

Methods

This exploratory study was a secondary, retrospective analysis of previously collected data from an Institutional Review Board-approved longitudinal parent study in a cohort of children with TBI. The enrollment process followed three main steps: Prescreening the census, screening the chart for study eligibility, and obtaining consent; assent was obtained whenever possible, based on the child's age and cognitive capacity. Children were considered eligible for enrollment into the parent study if they were previously healthy, had no history of TBI, were aged 5 days to 15 years at the time of injury, and were admitted for a mild, moderate, or severe TBI to the Pediatric Intensive Care Unit or Step-Down Unit at a university-affiliated level 1 trauma hospital. The parent study excluded children with prior diagnosed TBI, acquired brain injury (such an aneurysm) and/or developmental delay. The study team also did not enroll children who were likely to be progressing to brain death within 24 hours of hospital admission. Eligible individuals were enrolled if their parent or legal guardian completed enrollment paperwork, which included a blanket consent to provide multiple specimens whenever possible based on standards of care, over the course of their hospital stay (on days 1, 2, 3, and 5). Blood samples were collected whenever there was an existing point of access (e.g., indwelling intravenous catheter) or when/if a standard of care lab was being collected. No venipuncture outside of standard of care was obtained. Blood was collected using standard 4 mL ethylenediaminetetraacetic acid prepared tubes. Urine was collected via nursing personnel by access to a sterile indwelling urinary catheter, by free catch sterile collection into a sterile urine container, or, for young participants, into a diaper; the exact route for sample collection is unavailable at the time of this report. Urine and blood, when collected, were obtained at the same time points. Samples were collected between days 1 and 5, with differing frequency among participants, depending on if/when there was a clinical indication for blood draw and timing hospital discharge. All biospecimens were processed according to the manufacturer's instruction and stored in a -80° Celsius freezer until future biomarker analysis. In addition to the inclusion criteria for enrollment into the parent study, participants had to have blood and/or urine available on day 1 to be included in this secondary analysis.

Participant injury characteristics and clinical data were abstracted from electronic health record and data was stored in a Research Electronic Data Capture database. SPSS version 25 was used for data analysis, management, and cleaning. Descriptive statistics were generated to explore differences in biospecimen donation by type.

Results

Demographics

Of the 60 children enrolled in the parent study, 37 children had blood and/or urine collected at time-point 1 and were included in this secondary analysis. The majority of participants identified as white 75.7% ($n = 28$), non-Hispanic 86.5% ($n = 32$), and male 59.5% ($n = 22$). TBIs were primarily due to falls 56.8% ($n = 21$) and 94.6% ($n = 35$) were diagnosed as mTBI, defined as a Glasgow Coma Scale (GSC) score of 13-15. The most common structural alteration visible on imaging was a skull fracture (48.6%; $n = 18$), and the second most common was a subdural hematoma (35.1%; $n = 13$). See Table 1 for additional demographic and injury characteristics of the participants.

Table 1

*Demographics and Injury Characteristics (*n = 37*) of Consented Individuals.*

Variable	Categories (If Applicable)	*(n) %*
Sex	Male	(*n* = 22) 59.5%
	Female	(*n* = 15) 40.5%
Race	White	(*n* = 28) 75.7%
	Black	(*n* = 8) 21.6%
	Other	(*n* = 1) 2.7%
Ethnicity	Non-Hispanic	(*n* = 32) 86.5%
	Hispanic	(*n* = 5) 13.5%
Glasgow Coma Scale Severity	Mild (14-15)	(*n* = 35) 94.6%
	Moderate (9-13)	(*n* = 2) 5.4%
	Severe (3-8)	(*n* = 0) 0.0%
Age in Years, mean (range, standard deviation [SD])	5.5 years (mean range= 0-15; *SD* = 5.45)	
Mechanism	Fall	(*n* = 21) 56.8%
	Motor Vehicle Collision	(*n* = 3) 8.1%
	Abusive Head Trauma	(*n* = 6) 16.2%
	Other	(*n* = 7) 18.9%
Injury Type	Subdural Hemorrhage	(*n* = 13) 35.1%
	Epidural Hematoma	(*n* = 10) 27.0%
	Intracerebral Hemorrhage	(*n* = 1) 2.7%
	Subarachnoid Hemorrhage	(*n* = 8) 21.6%
	Extra Axial Hematoma	(*n* = 6) 16.2%
	Skull Fracture	(*n* = 18) 48.6%
	Cerebrospinal Fluid Leak	(*n* = 1) 2.7%
	Contusion	(*n* = 3) 8.1%
	Concussion With No Acute Intracranial Process	(*n* = 7) 18.9%

Sample Availability

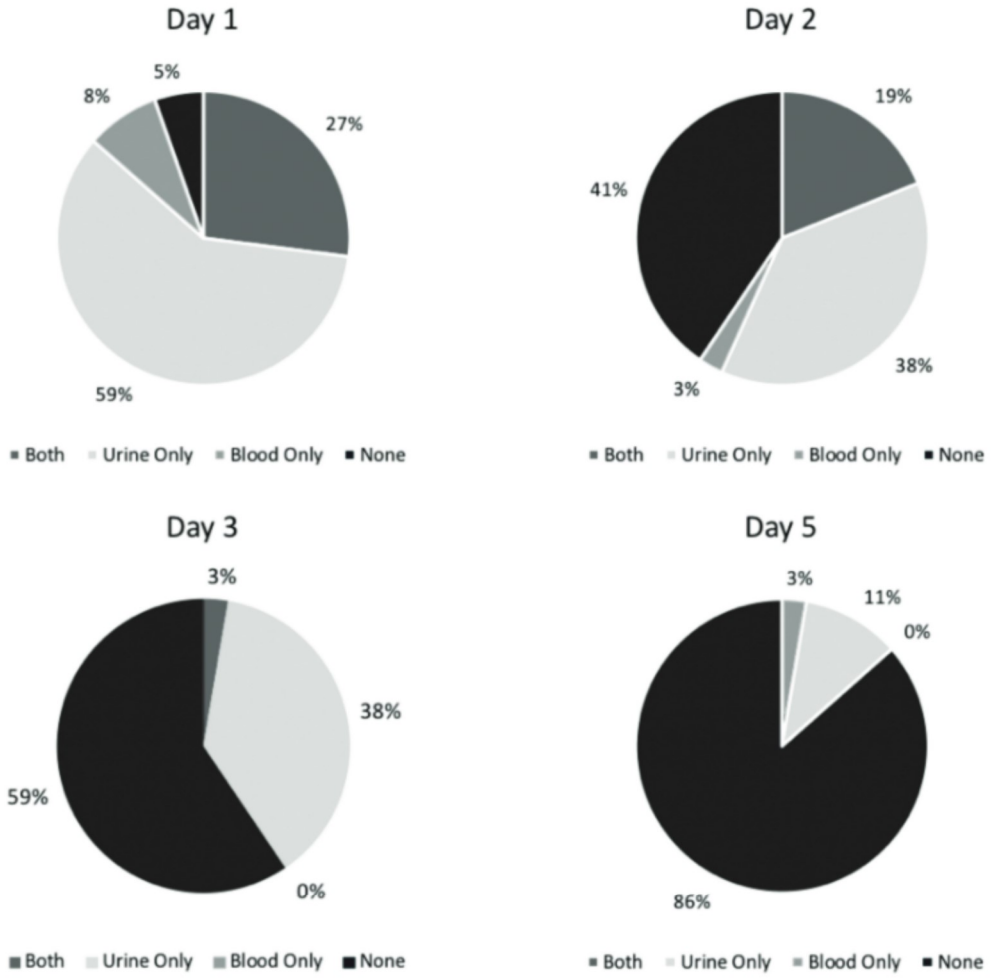
Whenever possible, urine and blood samples were collected on days 1, 2, 3, and 5. Notably, differences in TBI severity, clinical management, and discharge led to major differences between the number of blood and urine collections. On day 1, 2.46 times more urine samples were collected than blood samples (see Figure 1). By day 5, 13.5% of participants were still providing urine samples when compared to only 2.7% of participants who provided blood samples. Over the five-day period, 8.1% ($n = 3$) participants switched from giving blood only to giving urine only, and 18.9% ($n = 7$) switched from providing both blood and urine samples to only providing urine. Patients providing only blood or both blood and urine samples fell from 35% to 3% over the course of the five days, while patients providing only urine versus blood and urine fell from 86% to 11% over the same time-period. Discontinuation of urine collection was due to hospital discharge, whereas discontinuation of blood was based on IV removal or lack of need for blood-based laboratory tests.

Discussion

The present study is novel in that it is the first to demonstrate the availability of urine and blood samples when dovetailing collection of biospecimens for research purposes with clinical care. When feasible, researchers wishing to analyze biomarkers should consider trying to collect samples as part of standard of care to minimize risks to pediatric participants and burden to clinicians. Blood collection poses risk for in-

Figure 1

Breakdown of Participants Who Donated Blood, Urine, Both, or Neither on Each Day of Biospecimen Collection in the Parent Study



fection to participants and has the potential for specimen contamination or coagulation, which could lead to a need for repeat blood draws (Hall et al., 2013). Utilizing well-trained phlebotomy teams has been found to reduce risk of infection (Gander et al., 2009), but the resources necessary to support such staff is not always available. Likewise, there are risks associated with both urinary catheterization and sterile catch; considerations for infection risk, invasiveness, and time/resource utilization must be considered (Eckert et al., 2020; Kaufman, Sanci, et al., 2020; May, 2018). More importantly, understanding the availability of dovetailing research sample collection with clinical care may inform future study design to improve study enrollment, longitudinal retention, maximize biobanking success and subsequent expansion of the pediatric biomarker knowledge base.

The primary finding of this study was that, for pediatric TBI patients undergoing biomarker collection that dovetailed with standard of care, urine was more readily available when compared to blood. Many children who sustain a brain injury, such as a mild TBI, do not require IV access or blood-based laboratory tests. Likewise, as children recover from injury over the course of their hospitalization, IV lines are discontinued when no longer clinically indicated and the need for blood analysis to measure physiologic and homeostatic stability decreases over time; these clinical realities contributed to decreased rates of blood biobanking over time in the parent study. After invasive catheters were removed and blood test orders discontinued, parents and children were not asked to continue to provide non-standard of care blood samples. This decision considered a desire to avoid continuing painful procedures and to not overburden clinicians by asking them to assist in additional sample collection outside of the standard of care.

While this study utilized blanket consent that piggybacked biobanking with a standard of care, this approach is not always possible. Many research studies collect biological specimens outside of standard of care, which relies on individual consent to additional specimen collection procedures. In the parent study, urine was the most available for collection; the pediatric TBI patients had limited and highly variable use of IV lines and venipuncture for blood analysis as part of standard of care. Limited vascular access as part of standard of care means that studies wishing to collect blood in the pediatric TBI population may need to rely more on informed consent for study-specific venipuncture. Investigators wishing to prospectively collect blood for research purposes should consider published evidence surrounding factors that may impact blood draw success, study recruitment and retention of participants longitudinally. Available evidence suggests fear of needles is significant for pediatric patients, which highly impacts willingness to provide blood samples for biobanking purposes. This finding is evidenced by McMurty and teams (2015) study, where 63% of chil-

dren 6 to 17 years of age acknowledged a fear of procedures involving needles. Whereas another study found the self-reported fear of needles varied by age, with a rate of 68% in children aged 6 to 8, 65% in children aged 9 to 12, and 51% in children aged 13 to 17 (Taddio et al., 2012). Studies have also reported venipuncture and blood procurement influences parental consent in pediatric studies; 42% of participants whose parents did not consent to one study listed blood procurement as a reason, due to the pain involved during the procedure (Langley et al., 1998). Options to minimize needle fear or repeated non-standardized blood collections may include a detailed informed consent and assent document that addresses these risks in understandable terms and adoption of pain relief methods (e.g., numbing spray), which may reduce the impact of fear on biobanking success. Ultimately, blood collection can be a significant barrier to pediatric study enrollment as it can impact consent rates, increases risk to participants, and creates additional costs for studies. Yet, a viable option exists in the collection of non-invasive biologic samples, which are more likely to be met with more successful enrollment, retention, and collection (Gorodischer et al., 1994; Oerlemans et al., 2018; Ritchie et al., 2019).

Urine was the most easily accessible biospecimen in all severities and informal discussion with parents and children suggested that they did not find this type of collection objectionable. In this study, patients provided significantly more urine samples than blood samples. On the first day, 2.46 times as many urine samples were collected than blood samples, whereas on the fifth day of data collection, there were five times as many urine samples collected than blood samples. This result echoes the findings of another study, focusing on biomarkers in multiple sclerosis, which highlighted the use of urine in serial sampling because of its ease of collection (Dobson, 2012). Whether dovetailing sample collection with clinical care or collecting samples exclusively for research purposes, there are some practical advantages to urine over blood. For example, blood collection requires sample tubes containing anticoagulating compounds and ligand binding compounds to stabilize the blood components, while urine collection simply involves a sterilized sample (Lindsay & Costello, 2017). Urine collection methods have been validated in young neonates to identify biomarkers that promote care management (Ritchie et al., 2019) and in non-toilet-trained children in biological monitoring studies (Oerlemans et al., 2018). Thereby indicating the use of urine as an alternative to blood as a source of biomarkers could reduce costs, resolve these fears and parental apprehension, and lead to higher consent rates and ultimately enrollment into biological banks that can be used to support diagnostic and prognostic advances.

While dovetailing research specimen collection minimized risk to participants and healthcare provider burden, it resulted in limited biospecimens available for biomarker analysis because blood was not available unless an existing blood-based access was in place, or a venipuncture was performed as a part of the standard of care for treatment. Moreover, this secondary analysis is a single-site study with a small, homogeneous sample which included mostly white males; this limits the generalizability of the present study. Replication in larger, more diverse samples is needed. Due to the lack of blood versus urine samples in the mTBI patients, direct comparisons between serum and urinary biomarker levels were not possible. Continued efforts to compare the type, nature, and utility of biomarkers in blood versus urine are warranted. Future studies on procurement and analysis of urinary biomarkers would allow for potentially larger sample populations with greater power, and diagnostic and/or prognostic significance. Due to the practicality of using urine, additional research addressing the sensitivity and specificity for noninvasive diagnostic biomarkers is required. Additionally, there is minimal evidence regarding the most efficient methods to biobank pediatric samples when not collected as part of standard of care, as well as the feasibility and limits of collecting other biospecimens (e.g., cerebrospinal fluid; other tissues). Finally, replication is limited by the nature of retrospective secondary analyses. Larger prospective trials would be directly aimed at addressing the limitations of the present study.

Conclusions

Although there were limitations in this study, the study provides interesting early data that suggests collection of urine for future biomarker discovery are more readily available in pediatric TBI patients receiving standard of care. Considerations for the nature of sample collection should be considered, beyond what was included in this study. For example, factors that affect availability of samples as part of clinical care or willingness of children and their legal guardians to participate should be considered. Factors including the cost-effectiveness, infection risk, and acceptability to children and families must be considered (Eckert, et al., 2020; Kaufman, Knight, et al., 2020; Kaufman, Sanci, et al., 2020). Likewise, consideration for timing of urine collection and processing technique should be explored, as these have been found to impact biomarker data; formal evaluations of urine collection route are worth pursuing as the impact on biomarker analysis remains unknown (Thomas et al., 2010).

Overall, compared to blood-based biomarkers, the state of the science for urinary biomarkers is lagging and the need for reproducible protocols has been identified; Likewise, less is known about the normal human urinary proteome (Beretov, et al., 2014; Harpole et al., 2016). Current applications for urine are also more limited with the most common applications surrounding urinary health (Watson et al., 2016). Although there is a growing body of literature exploring urinary biomarkers of neurodegenerative disease (An & Gao, 2015).

References

An, M., & Gao, Y. (2015). Urinary biomarkers of brain diseases. *Genomics, Proteomics & Bioinformatics, 13*(6), 345–354. https://doi.org/10.1016/j.gpb.2015.08.005

Beretov, J., Wasinger, V. C., Schwartz, P., Graham, P. H., & Li, Y. (2014). A standardized and reproducible urine preparation protocol for cancer biomarkers discovery. *Biomarkers in Cancer, 6*, 21–27. https://doi.org/10.4137/BIC.S17991

Dadas, A., Washington, J., Diaz-Arrastia, R., & Janigro, D. (2018). Biomarkers in traumatic brain injury (TBI): A review. *Neuropsychiatric Disease and Treatment, 14*, 2989–3000. https://doi.org/10.2147/NDT.S125620

Davit, C. J., Hundley, R. J., Bacic, J. D., & Hanson, E. M. (2011). A pilot study to improve venipuncture compliance in children and adolescents with autism spectrum disorders. *Journal of Developmental and Behavioral Pediatrics, 32*(7), 521–525. https://doi.org/10.1097/DBP.0b013e3182245b09

Dobson, R. (2012). Urine: An under-studied source of biomarkers in multiple sclerosis? *Multiple Sclerosis and Related Disorders, 1*(2), 76–80. https://doi.org/10.1016/j.msard.2012.01.002

Duff, A. J. (2003). *Incorporating psychological approaches into routine pediatric venipuncture. Archives of Disease in Childhood.* Retrieved from www.archdischild.com

Eckert, L., Mattia, L., Patel, S., Okumura, R., Reynolds, P., & Stuiver, I. (2020). Reducing the risk of indwelling catheter-associated urinary tract infection in female patients by implementing an alternative female external urinary collection device: A quality improvement project. *Journal of Wound, Ostomy, and Continence Nursing: Official Publication of the Wound, Ostomy and Continence Nurses Society, 47*(1), 50–53. https://doi.org/10.1097/WON.0000000000000601

Gander, R. M., Byrd, L., DeCrescenzo, M., Hirany, S., Bowen, M., & Baughman, J. (2009). Impact of blood cultures drawn by phlebotomy on contamination rates and health care costs in a hospital emergency department. *Journal of Clinical Microbiology, 47*(4), 1021–1024. https://doi.org/10.1128/JCM.02162-08

Gorodischer R, Burtin P, Hwang P, Levine M, Koren G. (1994). Saliva versus blood sampling for therapeutic drug monitoring in children: Patient and parental preferences and an economic analysis. *Therapeutic Drug Monitoring, 16*(5), 437-443. https://doi.org/10.1097/00007691-199410000-00001

Hall, R. T., Domenico, H. J., Self, W. H., & Hain, P. D. (2013). Reducing the blood culture contamination rate in a pediatric emergency department and subsequent cost savings. *Pediatrics, 131*(1). https://doi.org/10.1542/peds.2012-1030

Harpole, M., Davis, J., & Espina, V. (2016). Current state of the art for enhancing urine biomarker discovery. *Expert Review of Proteomics, 13*(6), 609–626. https://doi.org/10.1080/14789450.2016.1190651

Howie, S. R. C. (2011). Blood sample volumes in child health research: Review of safe limits. *Bulletin of the World Health Organization, 89*(1), 46–53. https://doi.org/10.2471/BLT.10.080010

Kaufman, J., Knight, A. J., Bryant, P. A., Babl, F. E., & Dalziel, K. (2020). Liquid gold: The cost-effectiveness of urine sample collection methods for young precontinent children. *Archives of Disease in Childhood, 105*(3), 253–259. https://doi.org/10.1136/archdischild-2019-317561

Kaufman, J., Sanci, L., & Temple-Smith, M. (2020). What's the catch? Urine sample collection from young pre-continent children: A qualitative study in primary care. *BJGP Open, 4*(4), bjgpopen20X101060. https://doi.org/10.3399/bjgpopen20X101060

Kulbe, J. R., & Geddes, J. W. (2016). Current status of fluid biomarkers in mild traumatic brain injury. *Experimental Neurology, 275 Pt 3*(0 3), 334–352. https://doi.org/10.1016/j.expneurol.2015.05.004

Kurbatova, N., Garg, M., Whiley, L., Chekmeneva, E., Jiménez, B., Gómez-Romero, M., Pearce, J., Kimhofer, T., D'Hondt, E., Soininen, H., Kłoszewska, I., Mecocci, P., Tsolaki, M., Vellas, B., Aarsland, D., Nevado-Holgado, A., Liu, B., Snowden, S., Proitsi, P., Ashton, N. J., … Lovestone, S. (2020). Urinary metabolic phenotyping for Alzheimer's disease. *Scientific Reports, 10*(1), 21745. https://doi.org/10.1038/s41598-020-78031-9

Langley, J. M., Halperin, S. A., Mills, E. L., & Eastwood, B. (1998). Parental willingness to enter a child in a controlled vaccine trial. *Clinical and Investigative Medicine, 21*(1), 12–16.

Lindsay, A., & Costello, J. T. (2017, January 1). Realising the potential of urine and saliva as diagnostic tools in sport and exercise medicine. *Sports Medicine.* Springer International Publishing. https://doi.org/10.1007/s40279-016-0558-1

Lisowska-Myjak B. (2010). Serum and urinary biomarkers of acute kidney injury. *Blood Purification, 29*(4), 357–365. https://doi.org/10.1159/000309421

May O. W. (2018). Urine collection methods in children: Which is the best? *The Nursing Clinics of North America, 53*(2), 137–143. https://doi.org/10.1016/j.cnur.2018.01.001

McMurtry, C. M., Riddell, R. P., Taddio, A., Racine, N., Asmundson, G. J. G., Noel, M., Chambers, C. T., Shah, V., & HELPinKids&Adults Team. (2015). Far from "just a poke": Common painful needle procedures and the development of needle fear. *Clinical Journal of Pain, 31*(10), S3–S11. https://doi.org/10.1097/AJP.0000000000000272

Oerlemans, A., van Dael, M., Vermeulen, R., Russel, F., & Scheepers, P. (2018). Urine collection methods for non-toilet-trained children in biological monitoring studies: Validation of a disposable diaper for characterization of tebuconazole exposure. *Toxicology Letters, 298,* 201–206. https://doi.org/10.1016/j.toxlet.2018.09.018

Oris, C., Pereira, B., Durif, J., Simon-Pimmel, J., Castellani, C., Manzano, S., Sapin, V., & Bouvier, D. (2018). The biomarker S100B and mild traumatic brain injury: A meta-analysis. *Pediatrics, 141*(6), e20180037. https://doi.org/10.1542/peds.2018-0037

Ritchie D, Broadbent R, Medlicott N, Reith DM. (2019). In vitro validation of a method for neonatal urine collection and analysis. *BMJ Paediatric Open, 26*, 3(1), e000482. https://doi.org/10.1136/bmjpo-2019-000482.

Seol, W., Kim, H., & Son, I. (2020). Urinary biomarkers for neurodegenerative diseases. *Experimental Neurobiology, 29*(5), 325–333. https://doi.org/10.5607/en20042

Strimbu, K., & Tavel, J. A. (2010). What are biomarkers? *Current Opinion in HIV and AIDS, 5*(6), 463–466. https://doi.org/10.1097/COH.0b013e32833ed177

Taddio, A., Ipp, M., Thivakaran, S., Jamal, A., Parikh, C., Smart, S., Sovran, J., Stephens, D., & Katz, J. (2012). Survey of the prevalence of immunization non-compliance due to needle fears in children and adults. *Vaccine, 30*(32), 4807–4812. https://doi.org/10.1016/J.VACCINE.2012.05.011

Thomas, C. E., Sexton, W., Benson, K., Sutphen, R., & Koomen, J. (2010). Urine collection and processing for protein biomarker discovery and quantification. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 19*(4), 953–959. https://doi.org/10.1158/1055-9965.EPI-10-0069

Chaban, V., Clarke, G., Skandsen, T., Islam, R., Einarsen, C. E., Vik, A., Damås, J. K., Mollnes, T. E., Håberg, A. K., & Pischke, S. E. (2020). Systemic inflammation persists the first year after mild traumatic brain injury: Results from the prospective trondheim mild traumatic brain injury study. *Journal of Neurotrauma, 37*(19), 2120–2130. https://doi.org/10.1089/neu.2019.6963

Vos, P. E., Jacobs, B., Andriessen, T. M., Lamers, K. J., Borm, G. F., Beems, T., Edwards, M., Rosmalen, C. F., & Vissers, J. L. (2010). GFAP and S100B are biomarkers of traumatic brain injury: An observational cohort study. *Neurology, 75*(20), 1786–1793. https://doi.org/10.1212/WNL.0b013e3181fd62d2

Watson, J. R., Hains, D. S., Cohen, D. M., Spencer, J. D., Kline, J. M., Yin, H., & Schwaderer, A. L. (2016). Evaluation of novel urinary tract infection biomarkers in children. *Pediatric Research, 79*(6), 934–939. https://doi.org/10.1038/pr.2016.33

Yang, S. H., Gangidine, M., Pritts, T. A., Goodman, M. D., & Lentsch, A. B. (2013). Interleukin 6 mediates neuroinflammation and motor coordination deficits after mild traumatic brain injury and brief hypoxia in mice. *Shock (Augusta, Ga.), 40*(6), 471–475. https://doi.org/10.1097/SHK.0000000000000037