Fiscal Year:	FY 2024	**Task Last Updated:**	FY 01/27/2024
PI Name:	Lan, Li-i		
Project Title:	Ultra-Compact Urinary Calcium Measurement Device: Refinement and Application		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	Yes
Human Research Program Elements:	(1) **ExMC**:Exploration Medical Capabilities		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	03755	**Congressional District:**	2
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2020 HERO 80JSC020N0001-FLAGSHIP, OMNIBUS1 Human Research Program: Crew Health Appendix A; Omnibus1-Appendix B
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No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	1	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
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Flight Program:			
Flight Assignment:	NOTE: End date has been changed to 09/27/2025 per NSSC information (Ed., 7/12/24). NOTE: End date has been changed to 09/27/2024 per NSSC information (Ed., 11/15/23). NOTE: End date has been changed to 09/27/2023 per V. Lehman/JSC (Ed., 3/24/23). Original end date was 03/27/2023.		
Key Personnel Changes/Previous PI:	None.		
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Performance Goal Text:	
Task Description:	Slowing bone loss and preventing kidney stone formation are critical for successful spaceflight. The capability to track bone loss and kidney stone risk while in space would allow these risks to be monitored directly and would enable individualized countermeasure programs. At present, post-flight measurements are used to establish the effectiveness of the in-flight bone loss/kidney stone prevention program. A preventive approach would offer much greater operational flexibility, where ongoing in-flight measurements of countermeasure effectiveness would allow for adjustments in the countermeasure program during the flight. This approach could also be used during times when countermeasure equipment is broken, or when scheduling impacts the countermeasure program, to assess how this is affecting bone loss and kidney stone risk. In a previous NASA Omnibus project, we developed a prototype compact, low-power system to make urinary calcium measurements in space. This approach has proven to be feasible, but further development is needed to advance toward flight use. The objective of the current project is to improve this ultra-compact, robust, urinary calcium measurement system. This study will further a technology that may offer a personalized, preventive approach to bone loss and kidney stone prevention in space.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This research benefits life on Earth by developing a low cost, portable, and simple way to test urine calcium concentration in a remote setting or from the comfort of one's own home. This can be used for early detection of kidney disease. At-home testing of urinary calcium biomarkers allows individuals to monitor their kidney function regularly. This can help detect kidney disease at an early stage, thus enabling timely treatment and preventing further damage. A device with this portability and form factor promotes convenience. At-home testing of urinary calcium biomarkers is quick, easy, and can be done in the privacy of one's own home. This convenience can encourage more individuals to monitor their kidney function regularly. The simplicity of this device can also be cost-effective. At-home testing of urinary calcium biomarkers is less expensive than going to a healthcare provider for the same test. This cost savings can increase access to kidney health testing for individuals who cannot afford traditional healthcare services. This in turn has public health benefits. Regular monitoring of urinary calcium biomarkers can help identify populations at risk for certain health conditions, including kidney disease, and allow for targeted public health interventions. Overall, a low cost, simple, and portable device for measuring urinary calcium biomarkers can promote early detection of kidney disease, provide convenience and cost savings, and have significant public health benefits.
	TASK RESULTS: Reagent Recipe Investigation Result In our testing of different reagent recipes, we determined that potassium hydroxide (KOH) with potassium citrate (K-citrate) provided the best calibration range, compared to the other three recipes tested. This recipe was linear from 60 mg/L to 600 mg/L. Sodium hydroxide (NaOH) and K-citrate was linear from 100 to 400 mg/L, NaOH was linear from 200 mg/L to 400 mg/L, and KOH was linear from 150 mg/L to 400 mg/L. A clinically relevant range is approximately from 0 to 300 mg/L. Urinary calcium values in spaceflight are expected to be shifted higher to about 200-600 mg/L. The KOH and potassium citrate recipe satisfied our goal of achieving a calibration within a clinically relevant range, eliminating the need to dilute the sample to within the reagent calibration range in most expected cases. Optrode Redesign and Fabrication Result In our efforts to redesign the optrode for liquid reagent handling, we explored two primary designs. One was a “plunger-like” design where reagent was preloaded in the tube and urine was drawn into the same chamber, using a syringe-like drawing mechanism. The two liquids were then in direct contact and the reagent and sample mixed via diffusion. Another design we explored hastened the mixing of the reagent with the sample. This was called the “tube within a tube” design. This optrode contained preloaded liquid reagent in the annulus of the two tubes. A urine sample was drawn into the center tube by capillary action. Once the urine was sampled, the center tube was removed quickly, depositing the sample linearly along the interior of the optrode, while simultaneously creating turbulence that aided mixing of the sample with the reagent. Both ideas were prototyped and evaluated. Ultimately, the plunger concept was chosen for expanded fabrication and use in our validation study with human urine because the design was much simpler and could be fabricated in our required testing quantities with consistency. After we chose the plunger/syringe design, we tested two primary fabrication methods to create an airtight seal inside the polycarbonate tube with an inner rod. Method A used a heated tip to create a slight flare at the end of a 1 mm diameter polystyrene rod. Alternatively, Method B used a metal die with a spherical recess. This die was heated and a polystyrene rod was pressed into the recess to create a spherical tip. These slightly flared geometries created a seal when the 1 mm diameter polystyrene rod was threaded through a polycarbonate tube with a 1 mm internal diameter. Eleven units of each rod were created and used to measure a sample of 150 mg/L calcium standard. The repeatability of the output voltage was used as a metric of consistency. Method A was more consistent and was also faster to fabricate. Device Validation Study with Human Urine Result Preliminary assessment of the first 28 urine samples demonstrated poor linear voltage response against the clinically measured calcium concentration. The device had an R2 of 0.329 when a linear best fit line was applied to the data. For the device to be accurate, regardless of the final calibration equation used to convert the voltage into a calcium concentration, a strong linear response fit is needed. We paused urine collection and explored various sample preparation techniques and found that mixing the urine sample with the reagent in a test tube using a micropipette before drawing the solution into the optrode for reading provided the most consistent result. We used 450 microliters of reagent to 30 microliters of urine sample to maintain the correct reagent to sample ratio. The new mixing method on the subsequent 66 urine samples had an R2 of 0.868. The fact that mixing the samples led to more consistent results, and lower signal, was an unexpected result for us. It suggested a signal gradient along the length of the optrode tube, where signals close to the detector had a higher signal strength than those originating farther away. We investigated this behavior by measuring the signal attenuation in the

Task Progress:	sample using a spectrometer, and developing an optical model of the optrode. This revealed an unexpectedly high signal loss along the length of the optrode. Bench testing with urine and calcium standards confirmed the relationship between signal strength and distance from photodetector. This result means that poorly mixed samples, where the urine is close to the detector, will be biased to higher signals, which we observed in our testing. It also suggests that our optrode design is inefficient and a better design would involve a shorter tube with larger diameter. The influence of magnesium on magnitude of the residual was also investigated. In the previous NASA Human Exploration Research Opportunities (HERO) project, magnesium caused significant error in our dataset, because it can compete with calcium in its binding with calcein. Magnesium binding can be suppressed by elevating pH above 13. This is controlled in our system by increasing the pH of our sample using KOH. The plot shows little influence of magnesium on the residual, suggesting that we have good control over magnesium interference in our system. Under the best-case scenario, if we use the best fit line equation of our data as our calibration equation to convert voltage response into urinary calcium values, we can get an optimistic estimate of device accuracy in units of concentration. In operational use, a calibration equation would be determined ahead of time. Absolute percent error was calculated for each sample by finding the positive difference between the calcium concentration of the clinical lab values from those of the prototype values divided by the clinical lab values. We also expect only samples between 60 and 600 mg/L to be accurate on our system, based on the linear range established from our recipe investigation. Removing samples that fall outside that range, we get an average percent error of 14.41%. This is very close to our target of 10% error. An error of 14.44% may be comparable to the accuracy of the clinical lab analyzer. From our exploratory testing of the clinical analyzer accuracy, we found that the clinical analyzer had an average absolute percent error of 19.29%. This suggests that an accuracy level of approximately 20% may be clinically acceptable for diagnostic purposes, though more calcium standards would be needed to determine this with more confidence. A Bland-Altman analysis evaluated agreement between the clinical hospital analyzer and our device. The limits of agreement were 66 mg/L for a 95% confidence interval (i.e., +/-1.96*SD). This means that we can be 95% confident that the urinary calcium value measured with our prototype will be within 66 mg/L of the value that the hospital clinical analyzer would have measured. CONCLUSION: This project has successfully refined the ultra-compact urinary calcium measurement device from its embodiment in the previous NASA HERO project. The reagent recipe was successfully improved to expand the calibration to span a clinically relevant range. Although the sample preparation procedure was changed half way through the validation study, we have a clear idea of how to address the issues we encountered with an optrode redesign. Based on the work performed in this project, the device is now at a technology readiness level of 4 (TRL4: Component and/or breadboard validation in laboratory environment). Future steps to increase the device technology readiness level (TRL) include developing an operationally compatible way to collect a urine sample that is clean, safe, and reliable; redesigning the optrode to be shorter and have a larger diameter, which would improve the efficiency of the signal detection and improve the sample-reagent mixing, respectively; and validating the system in 0G. The device can be used with either 24-hour urine collection or first morning void urinary calcium concentration. Previous work from our group has shown that urinary calcium concentration measured from three consecutive first morning voids provides similar information to that of a 24-hour urine collection. Combining our device with this type of collection protocol may reduce the complexity of testing urine biomarkers in spaceflight. The development of this urinary calcium device can lay the groundwork for technologies and procedures to collect other relevant biomarkers from urine during spaceflight. This project directly addresses several NASA Human Research Roadmap gaps related to bone health and kidney stone prevention. [Note: For the related NASA HERO project, see "Ultra-Compact Device for Monitoring Bone Loss and Kidney Stone Risk" (PI: Buckey; Grant #80NSSC19K1632), Ed., 2/21/24.]
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