SLIPS-LAB—A bioinspired bioanalysis system for metabolic evaluation of urinary stone disease

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Urinary stone disease is among the most common medical conditions. Standard evaluation of urinary stone disease involves a metabolic workup of stone formers based on measurement of minerals and solutes excreted in 24-hour urine samples. Nevertheless, 24-hour urine testing is slow, expensive, and inconvenient for patients, which has hindered widespread adoption in clinical practice. Here, we demonstrate SLIPS-LAB (Slippery Liquid-Infused Porous Surface Laboratory), a droplet-based bioanalysis system, for rapid measurement of urinary stone-associated analytes. The ultra-repellent and antifouling properties of SLIPS, which is a biologically inspired surface technology, allow autonomous liquid handling and manipulation of physiological samples without complicated sample preparation procedures and supporting equipment. We pilot a study that examines key urinary analytes in clinical samples from patients with urinary stone. The simplicity and speed of SLIPS-LAB hold the potential to provide actionable diagnostic information for patients with urinary stone disease and rapid feedback for responses to dietary and pharmacologic treatments.

INTRODUCTION

Urinary stone disease affects ~19% of men and 9% of women during their lifetimes and imposes $5 billion in annual health care expenditures in the United States (1, 2). The recurrence rate of urinary stone is as high as 60 to 80%, highlighting the importance of preventive efforts. Current guidelines recommend metabolic evaluation of high-risk stone formers, such as patients with personal and family histories of urinary stones, obesity, and high blood pressure, with 24-hour urine collection to identify abnormal concentrations of minerals and salts that increase stone risk (3). Measurement of these solutes over 24 hours can diagnose potential underlying etiologies, guide dietary and pharmacologic interventions, and provide feedback for therapeutic monitoring (3–5). Twenty-four–hour urine testing is typically processed by commercial kits and laboratory services (e.g., Quest Diagnostics, Litholink, and UroRisk). The patient collects urine in a specialized container over a 24-hour period, and often, the container must be kept refrigerated throughout the collection. Following collection, an aliquot of the urine sample is shipped to a centralized clinical laboratory for measurement of urinary solutes. A clinical report is generated and sent to physicians in approximately 1 to 2 weeks; repeat collections are common and necessary for monitoring treatment response. Despite guideline recommendations from the American Urological Association and the European Association of Urology, 24-hour urine testing is not adopted widely in clinical practice because of the cost of testing, cumbersome collection procedures, and delay in obtaining the results (6, 7). There is an urgent need to develop rapid, low-cost, and effective diagnostic approaches for performing multiplex metabolic evaluation of high-risk populations and for providing rapid feedback on urinary responses to dietary or pharmacologic treatments at the point of care.

The advent of biologically inspired liquid-repellent technologies creates new opportunities in developing novel biomedical devices. Slippery Liquid-Infused Porous Surface (SLIPS) is a dynamic, molecularly smooth interface created by locking lubricating liquids in micro/nanostructured substrates (8). SLIPS is modeled after the Nepenthes pitcher plants that have evolved liquid-infused microtextured rims to capture insects (9). SLIPS exhibits many features that outperform state-of-the-art liquid-repellent coatings for biomedical applications, such as negligible adhesion to biological fluids, rapid and repeatable self-healing, resistance to biofouling, and adaptable liquid repellency in response to mechanical stimuli (10–14). SLIPS has been demonstrated for coatings of medical devices to prevent thrombosis and biofouling, as well as improving the sensitivity of surface-enhanced Raman scattering detection (15–18). Unlike other bioinspired surfaces, SLIPS is capable of maintaining low contact angle hysteresis and pinning free for various fluids (including blood and urine) (8). These unique features open new possibilities of microfluidic manipulation of physiological fluids for biomedical diagnostic applications.

In this study, we report SLIPS-LAB (SLIPS Laboratory), a droplet-based bioanalysis system, for rapid evaluation of urinary analytes that predict stone risk to facilitate timely and actionable management of patients with high-risk stone. We investigate mechanisms for liquid handling by the design of SLIPS-LAB. Taking advantages of the small contact angle hysteresis and contact line pinning-free properties of SLIPS, we establish liquid handling procedures, including sample loading, volume metering, droplet transportation, reaction time control, and analyte detection, and design a multiplex SLIPS-LAB device. The device implements multiple colorimetric and enzymatic assays simultaneously for detecting key urinary stone–associated analytes, including calcium, citrate, uric acid, oxalate, and pH. We evaluate the performance of SLIPS-LAB in examining spot urine collection and urine samples from patients with stone undergoing clinical workup with 24-hour urine collection. The results are compared with reports from clinical laboratories to evaluate the feasibility of...
SLIPS-LAB for rapid measurement of solutes associated with urinary stone disease.

RESULTS
Design of SLIPS-LAB for urinary stone disease metabolic workup
SLIPS-LAB is designed for accomplishing a urinary stone disease metabolic workup in 30 min (Fig. 1A). Design and assay requirements include direct handling of urine samples without preprocessing, implementing multistep bioanalytical procedures, and detecting multiple urinary stone–associated analytes in a single test. The SLIPS-LAB device is fabricated by polydimethylsiloxane (PDMS) molding of three-dimensional (3D) laser-machined structures and infusing hydrophilic hydroxy-terminated PDMS lubricant (contact angle $\theta = 76.2^\circ$) into the PDMS structure to create SLIPS coatings (fig. S1). We designed SLIPS-LAB to handle various types of fluids, transport liquid autonomously without an external pump or a power source, and perform multistep biochemical assays in a single device (Fig. 1B). Fluid sampling was performed by dipping the bottom inlet of the lubricated channel directly into the fluid reservoir or loading the fluid to the top inlets by capillary force. Droplet transportation is driven by a net force generated from the Laplace pressure gradient, which is inspired by shorebirds, spider silk, and cactus (19–21). Droplet transportation is enabled by the bioinspired surface technology, SLIPS, which is contact line pinning free and has extremely low contact angle hysteresis (13), for minimizing the retention force. Multistep biochemical reaction procedures were engineered into the SLIPS-LAB device by adjusting the channel design, which controls the reagent volume, the reaction sequence, and the delay time between reactions. The system does not require specialized instruments for detection, and the results of enzymatic and colorimetric assays can be rapidly quantified using a desktop scanner or a cell phone.

SLIPS-LAB liquid handling
We first demonstrated SLIPS-LAB for liquid handling of various viscous fluids and biological fluids (Fig. 1C and fig. S2). This capability is important because reagents and samples may have diverse viscosities and properties (22). The viscous fluids included water, milk, juice, glycerol, syrup, and honey. These fluids cover a large range of viscosity (1 to ~5000 centipoise) and various liquid handling applications, such as drug screening, environmental monitoring, and food safety (table S1). The biological fluids included urine, saliva, tracheal aspirate, plasma, and whole blood. The fluids were loaded in the bottom inlets (Fig. 1C, $t = 0$ s), and the device was placed horizontally. The droplet transportation process was initialized when the air hole was unsealed. Droplet movement was induced by the channel geometry without external pump, power, or control (movie S1). Droplets were transported from the bottom inlets toward the reaction chambers (Fig. 1C, $t = 6$ s). Urine samples required less than 10 s to reach the reaction chamber. The samples were subsequently mixed in the chambers (Fig. 1C, $t = 670$ or 92 s). Droplet transportation was robust, and there were no visible residues or traces on the SLIPS-coated channels for all fluids.

Working principles of SLIPS-LAB
We investigated the working principles of SLIPS-LAB for performing important liquid handling procedures including volume metering, droplet transportation, reaction time control, and mixing for biochemical analysis (Fig. 2A and movie S2). We first established two mechanisms of liquid sampling with a large range of volume (Fig. 2A, step 1). To sample a small volume, capillary force was sufficient to trap the liquid in a punched hole of the device when the sample passed through the hole (Fig. 2B and movie S3). The punched holes, which served as the top inlets, controlled the sample volume (1 to 15 µl) by the hole diameter and height (Fig. 2G and fig. S3). This volume range represents fluid samples with a dimension smaller than the capillary length, $l_c = (\gamma_{LV} / \rho g)^{0.5}$, where $\gamma_{LV}$ is the interfacial tension of the liquid–vapor interface, $\rho$ is the liquid density, and $g$ is the gravitational acceleration. The capillary length is approximately 2.7 mm for water (the cubic root of 15 µl or mm$^3$ is ~2.7 mm). To sample a large volume (i.e., dimensions larger than $l_c$), we designed a mechanism by drawing liquid from the reservoir (Fig. 2C and fig. S4).

![Fig. 1. Slippery Liquid-Infused Porous Surface Laboratory.](image-url)
Figure 2. Working principles of SLIPS-LAB. (A) SLIPS-LAB performs common biochemical procedures, including volume metering, liquid handling, reaction time control, and detection. Images are representative of two independent experiments. Scale bars, 2 mm. (B) Loading and metering of small-volume liquids in the top inlet by capillary force. (C) Sampling of large-volume liquids in the bottom inlet with air pressure by sealing the air hole. (D and E) Liquid transportation driven by the geometry-induced force imbalance on SLIPS, which has low contact angle hysteresis. The loading speed can be adjusted by the channel geometry to control the reaction sequence and the delay time for multistep reactions. (F) Reactions occur after merging and mixing of droplets in the reaction chamber. (G and H) Evaluation of volume metering for small-volume (<15 μl) and large-volume (>15 μl) liquids. D is the top inlet diameter. H is the dipping height of the liquid. (I) Controlling the loading time by the converging angle and channel thickness. T is the thickness of the channel. The loading time can be adjusted from a few seconds to ~4 min. Data represent mean ± SEM (n = 10 to 30). Photo credit: Hui Li (The Pennsylvania State University).

The device was dipped vertically into the reservoir with the air hole opened (23). The air hole was then sealed, and liquid droplets were trapped in the channel by air pressure when the channel was removed from the liquid reservoir. By engineering the dipping height and channel thickness, this approach was demonstrated for handling fluids in the range of 10 to 50 μl (Fig. 2H). These ranges (both small volume and large volume) were sufficient for implementing all biochemical assays in this study. The sampling and volume metering processes were repeatable and were insensitive to the converging angle (0° to 20°) of the loading channels (fig. S5).

Autonomous droplet transportation was demonstrated in SLIPS-LAB (Fig. 2A, step 2, and fig. S6). We designed a converging channel geometry to create droplet motion (Fig. 2D). The geometry of the channel modulated the Laplace pressure and the projected area of the droplet. In the converging channel, the droplet experienced a differential force between the sides, and a nonzero net force was created to drive the droplet toward the narrow side of the channel (Fig. 2E). This transportation mechanism, however, does not work with a typical PDMS microchannel (e.g., the same channel without the lubricant) because of the large contact angle hysteresis (24). Contact angle hysteresis, which is the difference between receding and advancing contact angles, represents a retention force against the droplet motion. In contrast, the contact angle hysteresis is markedly reduced to as few as 3° with SLIPS coatings (13), enabling autonomous droplet transportation. In our experiment, the loading speed was controlled by the converging angle and channel thickness (Fig. 2I and movie S4). The sample remained stationary in the straight channel (i.e., 0° converging angle) in the experiment.

Reaction sequence and delay time control for multistep biochemical reactions were realized by regulating the loading time in between droplets (Fig. 2A, step 3). Because the droplet loading speed depends on the channel geometry, the reaction delay time can be preprogrammed in the design of SLIPS-LAB. For instance, the converging angle and the channel thickness can be adjusted for reaction time control. By preprogramming the converging angle between 5° and 20° and the thickness of the channel between 0.75 and 3.0 mm, the loading time was tuned from 3 s to over 4 min (Fig. 2I). As a demonstration, a SLIPS-LAB device with two inlets connected to a reaction chamber was designed with a converging angle of 20° on the left channel and a converging angle of 5° on the right channel (Fig. 2A). In addition to water, reaction time control was demonstrated using human whole-blood samples (movie S5). Additional reaction time control, e.g., 30 min, could be implemented by resealing the air hole, which stopped the motion of the second droplet and opening the air hole again after the desired delay time (movie S6).

Fluid droplets generally stop at the end of the converging channel without entering the chamber (see movie S4 for an example). To address this issue, a 3D reaction chamber design was developed to guide the droplet from the channel to the reaction chamber for mixing and detection (Fig. 2A, step 4, and fig. S7A). The 3D reaction chamber, which has a reduction in height and a curved chamber boundary, facilitates wetting of the fluid on the reaction chamber surface and loading of the droplet. The design was demonstrated in different configurations experimentally (Fig. 2A and fig. S7B). Multiple air vents were incorporated in the design to avoid trapping of air between the droplets. Once the droplets are in contact, mixing of
fluids was governed by coalescence kinetics (25, 26). For instance, the sample in the top inlet was first mixed with the reagent loaded from the left inlet (Fig. 2A, t = 10 s, and Fig. 2F, step 1). A multistep reaction can be achieved with the second reagent loaded from the right inlet (Fig. 2A, t = 36 s, and Fig. 2F, steps 2 to 4). The liquid handling processes are repeatable, and the droplet transportation can be performed in the same device multiple times (figure S8) (27).

**SLIPS-LAB for multiplex detection of urinary stone–associated analytes**

We designed a six-plex SLIPS-LAB device for metabolic evaluation of urinary stone disease (Fig. 3, A to D, and fig. S9, A and B). The device conducts colorimetric and enzymatic assays in parallel for detecting calcium, citrate, uric acid, oxalate, and pH, which are among the most clinically relevant urinary analytes to assess stone risk and treatment response (Fig. 3E). The assay procedure for each analyte was designed according to the manufacturer’s instructions. For instance, the calcium assay requires mixing of the calcium detectors with o-cresolphthalein complexone and 8-quinolinol, whereas the citrate assay requires only a single master solution containing citrate lyase, horseradish peroxidase, and 10-acetyl-3,7-dihydroxyphenoxazinone. Uric acid and oxalate assays involve multiple steps with or without the requirement of a delay between the reactions. For example, the oxalate assay requires a minimum of 3 min between the first and second reactions (oxalate converter and enzyme mix). Furthermore, the assays require handling of fluid volumes that are over 30-fold apart (from 1.4 to 47 μl). Additional descriptions of the assays are described in figure S9C and table S2. These reactions result in products that can be detected colorimetrically.

We engineered the thickness of the channels (bottom inlets) to sample liquids of desired volumes. Two channel thickness values (1.5 and 3.0 mm) were included in the design, and the device sampled fluids from a laser-machined reservoir array that has a height of 3 mm (190 μl per well). The sample volumes drawn were 30 and 47 μl based on our calibration (Fig. 2H). The converging angle was adjusted between 5° and 20° to control the droplet loading time. For the calcium, citrate, and uric acid assays (3 mm thickness), the droplets reached the reaction chambers in ~3 s. For the oxalate, pH, and control assays (1.5 mm thickness), the droplets reached the reaction chambers in ~6 s. An exception is that the master solution for oxalate detection was loaded in ~220 s by controlling the converging angle to implement the 3-min delay time required for the reaction (Fig. 2I). In addition, the volume of the top inlet was controlled by the diameter of the punch hole (1.5 or 2.0 mm) and the thickness of the top PDMS layer (3.0 and 2.0 mm). The diameter, height, and location of the top inlets were optimized to sample liquids (Fig. 3C and fig. S9B). Liquid handling with the six-plex SLIPS-LAB device was demonstrated (Fig. 3D and movie S7). Droplet transportation was initiated when the air holes were unsealed. The droplets were transported according to the design (Fig. 3D, boxes 1 and 3). For the oxalate assay (Fig. 3D, box 2), as an example, the sample in the right inlet was first loaded and mixed with the reagent.

![Image](image-url)

**Fig. 3. The design of a multiplex SLIPS-LAB for urinary stone disease metabolic workup.** (A to C) A six-plex SLIPS-LAB for detecting urinary stone–associated analytes. Top and side views of the device are shown in (A) and (B), respectively. T1 and T3 indicate the thicknesses of the bottom inlets (channel thickness), T2 and T4 indicate the thicknesses of the top inlets (PDMS thickness), T1, T2, T3, and T4 can be adjusted independently to control the sample and reagent volumes. Dotted circles indicate the air holes. Asterisks in (B) indicate positions of the top inlets. Zoom-in views of the top inlets are shown in (C). Color dyes are loaded in the device for visualization. Scale bars, 5 mm (A and B) and 1 mm (C). (D) Operation of the six-plex SLIPS-LAB device. Box 1 indicates loading of reagents for calcium, citrate, and uric acid detection. Box 2 indicates loading of reagents for oxalate detection, which requires two steps and a time delay of at least 3 min between the reactions. Box 3 indicates droplet transport for pH detection and control. (E) Reagents, volumes, and loading times of SLIPS-LAB inlets for urinary stone metabolic workup. (F) Calibration of the colorimetric and enzymatic assays for urinary stone–associated analytes. Data color (red, green, and blue) represents the RGB element used in the image. Data represent mean ± SEM (n = 3), a.u., arbitrary units. (G) Detection of urinary stone–associated analytes in a spot urine sample by SLIPS-LAB. The results are compared with the data obtained by the manufacturer-recommended manual procedures in 96-well plates (standard method). Data represent mean ± SEM (n = 3 for SLIPS-LAB and n = 2 for standard method).
in the top inlet. The top inlet was positioned to the right to facilitate the merging of the urine and the converter. Agitation by gently shaking the device was performed to speed up the reactions. The volume and inlet position for each reagent are shown in Fig. 3E.

The colorimetric assays were measured using a desktop scanner to demonstrate SLIPS-LAB for point-of-care diagnosis without bulky supporting equipment and a centralized laboratory. These biochemical assays were calibrated using SLIPS-LAB (Fig. 3F). The changes of the RGB (red, green, and blue) intensity values and the most sensitive color element or the combination of the sensitive color elements were determined for each assay (fig. S10). The calcium, uric acid, and citrate assays were represented by the green element, and the oxalate assay was represented by the blue element. These color elements were consistent with the recommended wavelengths for absorbance measurement. Regression analysis was performed to determine a calibration curve for each assay (fig. S11). The pH value was determined by a combination of red and green elements.

**SLIPS-LAB for spot urine tests**

We evaluated the performance of SLIPS-LAB using a human urine sample collected from a healthy volunteer at a single morning time point (Fig. 3G). The results were compared with data obtained using standard manual procedures recommended by the manufacturers. For calcium, citrate, uric acid, and oxalate, a calibration curve was created for each assay, and the measurement was performed using 96-well plates (fig. S11). The pH value of the urine sample was measured using pH strips and determined by comparison with the reference chart (fig. S12). The measurements were performed by SLIPS-LAB in parallel (fig. S13). The results from SLIPS-LAB and 96-well plates were compared quantitatively. The data suggested excellent analytical agreement between the two platforms (fig. 3G). The sample had relatively high levels of uric acid and oxalate, and the pH value was close to 8, while calcium and citrate fell in the normal concentration ranges based on the daily average of healthy individuals. The relatively high levels of uric acid and oxalate may be due to diet or may reflect low water intake around the time of spot urine collection. This is in contrast to 24-hour urine collections that represent low water intake around the time of spot urine collection. The patients collected 24-hour urine samples with containers supplied by a third-party company and delivered to Quest Diagnostics for routine processing and testing. Two aliquots from the remaining discarded sample were analyzed by SLIPS-LAB at two different sites (Penn State and Stanford/VAPAHCS). We then compared the performance of SLIPS-LAB for measurement of

**Clinical application of SLIPS-LAB for metabolic evaluation of urinary stone disease**

We investigated the ability of SLIPS-LAB to measure concentration of solutes in 24-hour urine samples collected from recurrent stone formers (Fig. 4A). With institutional review board approval, 24-hour urine samples (n = 15) were collected from patients at the Kidney Stone Clinic at Veterans Affairs Palo Alto Health Care System (VAPAHCS). The urine samples were collected as part of clinical care to identify etiologic factors, guide treatment options, and monitor patient compliance of dietary and pharmacologic intervention. The patients collected their 24-hour urine samples with containers supplied by a third-party clinical diagnostic service (Quest Diagnostics) and submitted their samples to the clinical laboratory at VAPAHCS. After measuring the total volume from the 24-hour urine sample, an aliquot was sent to Quest Diagnostics for routine processing and testing. Two aliquots from the remaining discarded sample were analyzed by SLIPS-LAB at two different sites (Penn State and Stanford/VAPAHCS). We then compared the performance of SLIPS-LAB for measurement of

![Image](https://example.com/image.png)

**DISCUSSION**

This study demonstrates a bioinspired bioanalysis platform for rapid, point-of-care diagnostics of urinary stone disease. The ultra-repellency of SLIPS, as indicated by a small contact angle hysteresis, significantly reduces the friction and enables autonomous droplet transportation
of viscous fluids and biological fluids. SLIPS-LAB represents the first demonstration of hydrophilic slippery interfaces for autonomous droplet manipulation in microfluidics. This capability eliminates bulky supporting equipment, such as pipettes, pumps, and pressure sources that are required in many microfluidic devices. Compared to other fluid manipulation mechanisms (32, 33), SLIPS-LAB allows liquid handling procedures without the requirement of external power or control. Furthermore, microfluidics, because of the large surface-to-volume ratio, is highly prone to biofouling and clogging of channels (34). Because SLIPS-LAB is antifouling, pinning free, and molecularly smooth, it is able to avoid adhesion of target analytes on the channel surface and allows manipulation of physiological fluids, such as urine and blood. The exceptional antifouling property of SLIPS compared to other bioinspired surface technologies, which are often susceptible to fouling over prolonged contact with biological fluids (16), allows robust operation of SLIPS-LAB for clinical diagnostics. As demonstrated in this study, SLIPS-LAB droplet manipulation was reliable after repeated usage and long reaction delay. SLIPS-LAB, which combines these unique characteristics, represents a versatile platform for multiplex metabolic evaluation and other point-of-care diagnostic applications.

In this study, we design SLIPS-LAB for performing multiplex biochemical analysis and for metabolic evaluation of urinary stone disease. Unlike previous biomedical applications of SLIPS, which have focused primarily on surface coatings for catheters, tubing, and electrowetting-based devices (15, 17, 35–37), SLIPS-LAB is the first comprehensive microfluidic platform based on SLIPS for in vitro diagnostics directly from clinical samples. SLIPS-LAB performs liquid handling procedures, including fluid sampling, volume metering, droplet transportation, and reaction control, for biochemical assays with minimal external control and supporting equipment. The liquid handling procedures were established by the optimization and calibration of the device. The versatility, scalability, and low-cost features of SLIPS-LAB for performing multiplex analysis were demonstrated by engineering a six-plex device. The top and bottom inlets were designed to handle a large range of fluid volume for various biochemical assays. Enzymatic and colorimetric assays were implemented on SLIPS-LAB for detecting urinary calcium, citrate, uric acid, oxalate, and pH. The colorimetric readouts were measured using a commercial desktop scanner, which is low cost and easily accessible. These properties will facilitate the implementation of SLIPS-LAB for bioanalysis in nontraditional health care
settings without direct access to centralized clinical laboratories, such as remote clinics, local pharmacies, and nursing homes. The reusability of SLIPS-LAB may create unique opportunities for special applications that require repeated measurements in resource-limited settings such as long-duration space travel.

SLIPS-LAB successfully detected solute concentrations in clinical urine samples and captured metabolic profiles of patients compared to established clinical laboratories. Because these measurements were performed on urine samples from high-risk stone formers, the results support the feasibility of SLIPS-LAB for rapid, near-patient urine testing for metabolic evaluation of stone formers who are being evaluated for the first time or who are being monitored for treatment responses. The size, speed, and point-of-care nature of SLIPS-LAB may open new opportunities in on-demand testing and monitoring of urinary analytes, which address some of the major limitations of 24-hour urine testing. In the future, an extended panel that includes additional analytes (e.g., sodium, sulfate, and creatinine) will expand the scope of urine measurements that can be accomplished by SLIPS-LAB. The user interface of SLIPS-LAB can also be simplified, including sample loading, reagent well design, and integration with smartphone-based detection, and combined with other microfluidic modalities to facilitate the implementation of SLIPS-LAB in point-of-care settings.

By providing rapid, multiplex testing of a panel of urinary stone-associate analytes using spot urine samples, SLIPS-LAB may potentially transform metabolic evaluation and clinical management of urinary stone disease. Stone formers may check their spot urine samples at home in response to a specific treatment response. The size, speed, and point-of-care nature of SLIPS-LAB are being evaluated for the first time or who are being monitored for treatment responses. The device was bonded with a glass slide or a laser-machined acrylic sheet with double-sided tape.

**Droplet transportation in SLIPS-LAB**

Autonomous droplet transportation was demonstrated using viscous fluids and biological fluids. Unless otherwise specified, distilled water was applied for droplet transportation demonstration. Food products, including food dye, milk (1%) and fruit juices (100%) grape juice), maple syrup (Coombs Family Farms), and honey (100% pure honey, Buckwheat, Weis), were purchased from a grocery store (Weis Markets, State College, PA, USA). Glycerol was obtained from Fisher Bioreagents (153421). The spot urine sample was collected from a healthy volunteer and stored at 4°C. Saliva was freshly collected from a healthy volunteer before the experiment. Human whole-blood samples with citrate phosphate dextrose as anticoagulant were purchased from BioVIT. To isolate plasma, human whole blood was centrifuged at 200g for 20 min. The deidentified tracheal aspirate sample was obtained from Penn State Milton S. Hershey Medical Center. To perform the experiment, the samples (45 μl each) were loaded at the bottom inlets with the device tilted at an angle of ~45°. Then, the air hole was sealed by tape. To initiate droplet transportation, the device was placed flat on a table with the tape removed. The samples were loaded into the reaction chambers and mixed.

**Fluid sampling and volume metering**

To sample a large volume (>15 μl), the bottom inlets of SLIPS-LAB were dipped into the fluid reservoir (laser machine wells or a petri dish) with a known height. The device along with the fluids was drawn from the reservoir after the air channel was sealed with tape. To calibrate the fluid volume, the liquid was pipetted out and measured on an analytical balance (XS205, Mettler Toledo). The volume was then calculated based on the density of the fluid. To sample a small volume (<15 μl), top inlets were designed to retain fluids by capillary force. The fluid was soaked into a piece of tissue paper (Kimtech Science, 05511) and moved over the inlets with a tweezer (movie S2). The same procedure could also be performed using a pipette or a cotton swab. To calibrate the sample volume, the liquid in the inlets was pipetted out and measured on the balance. For small-volume samples (<2 μl), multiple samples were collected and measured together to improve accuracy.

**Quantitative study of the loading process**

To study the droplet transportation process and control the loading time, the converging angles of the channels were adjusted from 0° to 20° with an interval of 5°. The thicknesses of the channels were 0.75, 1.5, and 3.0 mm. The loading time was defined as the time for the sample reaching the reaction chamber. For a slower loading speed (e.g., a converging angle <5°), the loading time was sensitive to the fabrication variation, which resulted in a large batch-to-batch discrepancy. These designs were not considered. To obtain a longer delay time, the air hole could be resealed to stop droplet transportation and reopened after the desired delay time.

**Design of SLIPS-LAB for multiplex detection**

The SLIPS-LAB device for urinary stone disease metabolic workup consisted of six units. Two diameters of the top inlets (1.5 and 2.0 mm)
were included in the same device. The PDMS thicknesses (i.e., heights of the top inlets) were 2.0 and 3.0 mm. The top inlets were positioned either at the center of the chambers or near one of the channels. The converging angle of the channel was 5° or 20°. The thickness of the channel (i.e., bottom inlet) was 1.5 or 3.0 mm. The microwell array was fabricated by bonding a laser-machined acrylic sheet with a thickness of 3 mm on a glass slide. The diameter of the microwell was 10 mm. The liquid volume was 190 μl in each microwell. These values were calibrated and optimized for performing the assays in this study and can be modified easily for other biochemical assays.

**Detection of urinary stone–associated analytes**

Enzymatic and colorimetric assay kits were used to measure metabolic profiles in urine. The calcium assay was purchased from Cayman (701220). The uric acid assay was purchased from BioAssay Systems (D1UA-250). Uric acid (Sigma-Aldrich, U2625-25G) dissolved in 0.1 M tris-HCl (pH 7.2) was used for calibrating the assay kit. The citrate and oxalate assays were purchased from Sigma-Aldrich (MAK057 and MAK179, respectively). The pH indicator was purchased from MsLavenda.com. The pH buffer, ranging from 5.06 to 7.61, was prepared by mixing citric acid (0.1 M) and disodium phosphate (0.2 M) (40). The ‘M buffer (tris-HCl and magnesium sulfate) was applied to obtain a pH value of 8.21. The pH values were determined by a pH meter (HI 2210, HANNA instruments).

Calibration experiments were performed using SLIPS-LAB and a microplate reader (FlexStation 3, Molecular Devices) following instructions from the manufacturers. The calibration samples were obtained from the assay kits or pH buffers described above. For SLIPS-LAB, mixing was performed manually by gently agitating the device for 5 min and incubating it for 10 min at room temperature in a dark environment. The results were measured using a desktop scanner (MX660, Canon). The RGB intensities were measured in a region of 11 × 11 pixels in the reaction chamber. Each assay was scanned three times to measure the RGB intensity values. The most sensitive color element was chosen for detection.

**Spot urine test by SLIPS-LAB**

The performance of SLIPS-LAB for a spot urine test was compared with manual procedures in 96-well plates. The volunteer’s urine sample was collected in the morning. The urine sample was collected from a healthy volunteer and stored at 4°C. The sample volume was approximately 40 ml. The metabolic profile was measured using manual procedures in 96-well plates and SLIPS-LAB in parallel. The analyte concentrations were estimated based on the calibration curves determined for both methods.

**Urinary stone disease metabolic workup of clinical samples**

A validation study was performed using SLIPS-LAB for detecting urine samples from patients with urinary stone. The urine samples were collected as part of an exempted protocol by the Stanford University Institutional Review Board (IRB). Patients undergoing 24-hour urine collection were recruited in this study. The samples were submitted to the clinical laboratory at VAPAHCS. The sample was aliquoted into three tubes with at least 50 ml in each tube. One of the tubes was sent to the central laboratory (Quest Diagnostics), following the standard procedure. The other tubes were sent to research laboratories at the Pennsylvania State University (P.K.W.) and the Stanford/VAPAHCS (J.C.L.) to perform the tests by SLIPS-LAB independently. Each sample was tested in triplicates by SLIPS-LAB. The clinical reports from Quest Diagnostics were compared with the results obtained from SLIPS-LAB.

**Statistical analysis**

The clinical data were analyzed using linear regression analysis, Bland-Altman plots, and heat maps. Regression analysis was performed using GraphPad Prism. Outliers were determined using the Modified Thompson Tau method. In particular, the method determines data with a standardized residual over 2 and inconsistent with its neighbors. The outliers were highlighted in red in the Bland-Altman plot and were not shown in the regression analysis. To analyze the metabolic profile, the solute concentrations were normalized for each assay and presented in the heat maps.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/21/eaba8535/DC1

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