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$\Delta t \approx 45 \text{ s}$

Gas-phase electrochemical detection

$\text{NH}_4^+ + \cdot\text{OH} \rightleftharpoons \text{NH}_3^+ + \text{H}_2\text{O}$

$\sim 15 \mu\text{L}$ whole blood
Point-of-care analysis of blood ammonia with a gas-phase sensor

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Abstract

Elevated blood ammonia (hyperammonemia) may cause delirium, brain damage, and even death. Effective treatments exist but preventing permanent neurological sequelae requires rapid, accurate, and serial measurements of blood ammonia. Standard methods require volumes of 1 to 3 ml, centrifugation to isolate plasma, and a turn-around time of two hours. Collection, handling, and processing requirements mean that community clinics, particularly those in low resource settings, cannot provide reliable measurements. We describe a method to measure ammonia from small-volume whole blood samples in two minutes. The method alkalizes blood to release gas phase ammonia for detection by a fuel cell. When an inexpensive first-generation instrument designed for 100 µl of blood was tested on adults and children in a clinical study, the method showed a strong correlation (\(R^2 = 0.97\)) with an academic clinical laboratory for plasma ammonia concentrations up to 500 µM (16 times higher than the upper limit of normal). A second-generation hand-held instrument designed for 10-20 µl of blood showed a near perfect correlation (\(R^2 = 0.99\)) with healthy donor blood samples containing known amounts of added ammonium chloride up to 1000 µM. Our method can enable rapid and inexpensive measurement of blood ammonia, transforming diagnosis and management of hyperammonemia.

Key Words: Ammonia, hyperammonemia, point-of-care, whole blood, small-volume
plasma from the red blood cells by centrifugation, and then analyzes the plasma by an automated
method in which the enzyme glutamate dehydrogenase (GDH) converts α-ketoglutarate to
glutamate in a reaction that consumes ammonia in the plasma. The rate of decrease of the NADPH
concentration, which is measured spectrophotometrically, provides data for inferring the ammonia
concentration. This standard assay may fail from improper sample handling, hemolysis from
phlebotomy, or patient lipemia or bilirubinemia.

The shortcomings of the standard plasma ammonia assay have motivated efforts to develop
alternative methods. Ion selective electrodes can measure ammonia in plasma or whole blood, but
this requires sample volumes of several milliliters, a response time of 10 to 15 minutes, and
frequent electrode re-calibration. The ideal alternative would be a portable point-of-care device
that operates on small volumes of whole blood (e.g. a single drop of capillary blood) that is easily
available in any clinical setting. The PocketChem Blood Ammonia Checker (BAC) attempts to
meet these goals as a portable device that alkalizes a 20 µl blood sample and then quantifies
gaseous ammonia using a colorimetric test with bromocresol green. BAC has been used in
several research trials with humans, but despite being available for more than 25 years, it has
not been validated for clinical use. For example, a study comparing the performance of BAC with
the standard clinical ammonia assay showed an intraclass correlation coefficient of 0.80 and a
Deming linear regression correlation coefficient of only $R^2 = 0.705$, with a 13% false negative
rate, highlighting its inadequacy for clinical use.

More recently, researchers have reported a blood ammonia sensor that operates by
separating ammonium (NH$_4^+$) from blood using an ion exchange membrane, and then quantifying
the ammonium using a modified indophenol reaction with a colorimetric read-out. When tested
with blood samples with varying amounts of added ammonium chloride (NH$_4$Cl), the device
showed large errors of ±50 µM. Furthermore, the ion exchange process is slow (>20 min) and the
reagents required for the indophenol reaction have a limited shelf life.

Several investigators have attempted to develop a non-invasive breath test for blood
ammonia, but their devices and protocols have shown only weak correlation between breath and
plasma ammonia. Indeed, Kearney et al. have investigated breath tests to diagnose Helicobacter pylori stomach infection. High urease activity from the bacterium generates ammonium from the urea that is normally secreted into the stomach. Kearney et al. found consistently higher baseline breath ammonia levels from H. pylori infected patients, who constitute 50% of the world population. Thus, breath tests to measure blood ammonia are not clinically feasible in their current form.

Here we report a method for measuring blood ammonia that alkalizes blood samples to
liberate gaseous ammonia for quantification with a gas-phase sensor. This technology enables
rapid measurements in less than 2 minutes from small-volume samples using an inexpensive,
commercially available amperometric sensor, avoiding multiple problems that plague standard
clinical assays. We describe the design and clinical testing of a first-generation device that
measures ammonia from 100 µl blood samples in patients with hyperammonemia. We then
describe a second-generation device using the same sensor technology to measure ammonia from
10-20 µl blood samples spotted onto disposable test strips. Our results provide proof-of-concept
for a real-time, portable ammonemia detector (PAD) akin to a glucometer.
Results

First-generation PAD

To assess feasibility for measuring blood ammonia with a gas sensor, we designed and assembled a first-generation portable ammonemia detector (PAD) (Figure 1a). The detecting element is a 3-electrode amperometric sensor, which is currently worn as an industrial safety badge for workers at risk for gaseous ammonia exposure (Figure 1b). The sensor functions as a miniature fuel cell. When ammonia diffuses into the anode side of the fuel cell, it reacts with H$_2$O to form ammonium hydroxide (NH$_4$OH), which promotes an I$_2$ disproportionation (redox) reaction that produces iodate (IO$_3^-$) and iodide (I$^-$). Iodide is electrochemically oxidized at the anode and ambient air O$_2$ is electrochemically reduced at the cathode, generating a current (Figure 1c). The current response is proportional to ammonia concentration in the gas phase. The fuel cell sells for $200 as a single unit, and contains a starch I$_2$ reservoir that provides a 24-month manufacturer-rated lifetime.

Figure 1: First-generation portable ammonemia detector (PAD). a) Instrument photo. Top: digital electrical current read-out; middle: fuel cell housing attached to cutting tool; bottom: sliding platform showing cap for glass vial; right inset: glass vial. b) Fuel cell gas sensor. Lines show position inside the instrument. c) Chemistry of ammonia measurement. Alkalization of the blood sample leads to equilibrium of NH$_3$ between blood and headspace. The fuel cell generates current proportional to headspace NH$_3$ concentration. d) Current vs. time trace for blood sample. Insertion of the glass vial rubber top through the cutting tool occurred at 80 seconds; current reached equilibrium 120 seconds after sample insertion.

First-generation PAD measured blood ammonia by interfacing the sensor with the headspace of an alkalized blood sample. Small glass vials with a rubber septum cap were pre-loaded with K$_2$CO$_3$. A 100 µl blood sample was injected through the septum and mixed thoroughly with the K$_2$CO$_3$. This process raises the pH and ionic strength of the sample, alkalizing the blood and converting ammonium to ammonia. Placement of the vial on a sliding platform in the instrument allows the cutting tool to puncture the septum cap, connecting the headspace of the vial to the anode side of the gas sensor while maintaining a gas-tight seal. The instrument responds with a current that rises until ammonia equilibrates between headspace and blood sample, reaching a plateau within 120 seconds (Figure 1d). The plateau current is proportional to the ammonia concentration in the headspace, and thus to the ammonia concentration in the sample. This detection method is highly specific for ammonia because it is the only volatile amine released upon alkalization of blood with K$_2$CO$_3$. Furthermore, results were insensitive to modest variations in the sample volume (data not shown) because the equilibrium headspace ammonia concentration is
a tiny fraction (less than 1%) of the blood ammonia concentration, and the fuel cell consumes only small amounts of ammonia.

First-generation PAD can measure ammonia in any aqueous sample, including plasma. We analyzed aliquots of frozen plasma sample from a healthy donor. A single sample was thawed, and ammonium chloride was added to aliquots of this sample in amounts ranging from zero to 600 µM. The instrument produced a linear plateau current response ($R^2=0.99$) across the entire range of ammonia concentrations with a slope of 2.5 nA per µM ammonia. A second aliquot analyzed on a different day produced very similar results, indicating consistent day-to-day performance (Figure 2a). Based on a cutoff for the signal-to-noise ratio of 10, the estimated Limit of Detection (LoD) for the first-generation PAD is 2 µM (see Methods). The upper limit of the normal range for healthy adults is 30 µM, and the standard clinical assay does not report values lower than 10 µM.

![Figure 2](image.png)

**Figure 2**: Performance of first-generation PAD with standard samples. **a)** Instrument response vs. amount of ammonium chloride added to a series plasma samples on two different days. The response is the plateau current produced by the instrument for each sample. **b)** Instrument response vs. amount of ammonium chloride added to a set of whole blood and plasma samples obtained from the same blood draw.

To compare whole blood and plasma ammonia levels, we analyzed a set of fresh whole blood and plasma standards from the same blood draw. Plasma was isolated immediately after whole blood collection and analyzed subsequent to whole blood analysis to minimize contribution from the reported instability of ammonia levels in whole blood. The instrument produced nearly identical response curves and y-intercepts for the whole blood and plasma samples (Figure 2b). Thus, the instrument displayed the same sensitivity and the same response to ammonia in whole blood and plasma.

The result in Figure 2b justified a comparison of the performance of PAD on whole blood to the standard clinical plasma ammonia assay. We enrolled patients in a clinical study that allowed the acquisition of both measurements from the same blood draw. Study participants consisted of 8 patients (ages 4 days to 76 yr) with cancer and inborn errors of metabolism (Table 1). Data were collected over a 6-month period without sensor adjustments or re-calibration of the instrument. Blood from each venipuncture was split in two, with one sample analyzed by PAD and the other sent to our clinical laboratory (Figure 3a). When the plateau current from PAD was plotted against
the clinical laboratory plasma ammonia result, the data showed a strong linear correlation ($R^2 = 0.97$) over a wide range of clinically relevant ammonia concentrations (Figure 3b). Thus, for diverse diagnoses and patient ages, the new technology applied to whole blood samples accurately predicted results from the clinical plasma ammonia test.

| Table 1. Clinical Validation of First-generation PAD |
|---|---|---|---|---|---|
| Patient | Age | Sex | Ethnicity | Diagnosis | Clinical burden of testing |
| 1 | 76 yr | Male | South Asian | Neuroendocrine tumor | >1-hour travel to testing lab or 2-day turnaround time |
| 2 | 73 yr | Female | Asian | Porto-systemic shunt | >1-hour travel to testing lab or 2-day turnaround time |
| 3 | 45 yr | Female | Hispanic | Ornithine transcarbamylase deficiency | >1-hour travel to testing lab 124 tests during 30-day hospitalization |
| 4 | 6 yr | Female | Hispanic | Organic acidemia | >1-hour travel to testing lab 4 tests during 3-day hospitalization Multiple attempts for vascular access |
| 5 | 5 yr | Male | Asian | Organic acidemia | 15 tests during 11-day hospitalization |
| 6 | 3 yr | Female | Hispanic | Organic acidemia | >1-hour travel to testing lab 11 tests during 7-day hospitalization Multiple attempts for vascular access |
| 7 | 16 mo | Female | Caucasian | Organic acidemia | 112 tests during 52-day hospitalization |
| 8 | 4 days | Male | Caucasian | Organic acidemia | 132 tests during 31-day hospitalization |

Figure 3: Comparison to the standard clinical ammonia assay. a) Schematic depiction of study design. A 1-3 ml sample from a venous draw was processed according to the standard protocol at the Stanford Clinical Lab to measure the plasma ammonia level. A second 0.1 ml sample from the same venous draw was analyzed by the first-generation PAD. b) The instrument response to the whole blood sample vs. the plasma ammonia level reported by the Stanford Clinical Laboratory.
Second-generation PAD

Blood ammonia measurements from the small volumes of blood obtained with capillary blood samples from finger, heel, or earlobe sticks would greatly improve patient care. We therefore designed a second-generation PAD for blood sample volumes in the 10-20 µl range (Figure 4). This instrument links the same gas sensor and circuit board in the first-generation PAD to a sensor housing designed for blood samples spotted onto a disposable test strip. The sensor housing was fabricated out of plastic polyvinylidene difluoride (PVDF) to minimize interference from NH₃ adsorption on the internal surfaces. The disposable test strips consist of a PVDF support for K₂CO₃-impregnated Ahlstrom 1281 filter paper. On one side of the strip, a nylon mesh covers the filter paper to facilitate blood spreading. One PVDF layer contains a hole that permits blood delivery to a microfluidic channel, and a second small hole that allows air to escape as blood spreads over the strip. On the other side of the strip, a large aperture in a second PVDF layer exposes the filter paper to the fuel cell when the strip is inserted into the instrument (Figure 4a). The sensor housing consists of a PVDF chamber with a slot for inserting a disposable test strip (Figure 4b). Measurements were performed by drawing blood from the subject into a heparinized capillary tube, inserting the test strip into a slot in the sensor housing (Figure 4c), and touching the capillary tube to the inlet of a microfluidic channel that protrudes from the sensor housing. Capillary action draws the blood onto the paper, where contact triggers the alkalization and releases ammonia into the headspace that is in contact with the anode of the fuel cell. To maintain sensitivity, the headspace is much smaller in this second-generation design. The release of ammonia generates a current response that peaks within 45 seconds (Figure 4d). The peak current was proportional to the headspace ammonia concentration and the ammonia concentration in the blood sample.

Figure 4: Second-generation PAD for 10–20 µl blood samples. a) Schematic depiction of disposable test strip assembly. Filter paper was coated with K₂CO₃, attached to a PVDF solid support and covered with nylon mesh. Four additional PVDF layers (with functions described in the text) were mated by ultrasonic welding. b) Schematic depiction of instrument components; c) Photo of instrument and test strip; d) Current vs. time trace for a 15 µl blood sample. Peak current occurred 45 s after spotting sample onto the test strip.
We evaluated second-generation PAD with a series of water samples adjusted with ammonium chloride to achieve concentrations from 0 to 1000 µM. Sample analysis consisted of 5 replicates performed with samples spotted onto test strips. Sample volumes were 10 to 20 µl, as determined after depositing the blood onto the test strip. The instrument responded linearly ($R^2 = 0.999$) across the range of ammonia concentrations (Figure 5a). The current responses of the first-generation and second-generation PAD were comparable despite the large reduction in sample volume because the sensor electrochemical consumes only a small fraction of the total ammonia in the sample. Next, we tested the instrument with a series of whole blood samples prepared from a healthy donor (with blood ammonia < 10 µM) by adding ammonium chloride to generate elevated ammonia levels (0 to 1000 µM). The instrument response was nearly identical for blood and water samples. Thus, the second-generation PAD can accurately measure the ammonia content of small-volume blood samples spotted onto disposable test strips.

To assess the instrument’s resolution of ammonia levels below 100 µM, we analyzed a second series of water-based standards with ammonia concentrations ranging from 25 to 100 µM. Nine replicate measurements were performed for each sample and the t-distribution was used to construct 95% confidence intervals. Based on these confidence intervals, the instrument can resolve ammonia levels differing in the 25 to 100 µM range (Figure 5b). The estimated LoD for the second-generation PAD is 4 µM (see Methods).

![Figure 5: Performance of the second-generation PAD with water and blood standards. a) The current response of the instrument vs. the amount of ammonium chloride added to water (black) or whole blood (red) across a range of 0 to 1000 µM with a slope of 1.4 nA per µM. Error bars for the water samples are 95% confidence intervals for five measurements; b) The current response of the instrument vs. the amount of ammonium chloride added to a second series of water samples across a range of 25 to 100 µM. Error bars are 95% confidence intervals for nine measurements.](image)

**Discussion**

We describe a portable ammonemia detector (PAD) utilizing an inexpensive gas phase ammonia sensor capable of measuring blood ammonia levels rapidly and accurately. In a clinical study, a first-generation PAD for 100 µl blood samples produced results highly correlated with our institution’s clinical laboratory test ($R^2 = 0.97$) over a clinically relevant range from normal (<30...
µM) to highly elevated (500 µM). A second-generation PAD with disposable test strips designed for 10-20 µl blood samples was sensitive and accurate for blood samples containing known concentrations of ammonium chloride up to 1000 µM.

Compared to the standard clinical assay, PAD confers speed (with results in less than 2 minutes) and simplifies workflow. The standard assay requires transportation of the sample on ice to a central clinical laboratory, centrifugation, and analysis of the plasma in a commercial machine, reporting results after 2 hours. Moreover, hemolysis of blood samples may interfere with the colorimetric assay, requiring a second phlebotomy and additional waiting time. Idiosyncrasies of current clinical ammonia testing may force patients to travel to large medical centers to obtain reliable test results. In our clinical study, Patients 1 and 2 managed hyperammonemia as outpatients, but their local community hospitals failed to provide accurate results. However, driving from their homes to and from our medical center involved at least 2 hours of travel time (Table 1). Large commercial laboratories closer to their homes offered accurate testing, but with a 2-day turn-around for results. Compared to the standard clinical assay, PAD can provide results for patients and providers at the point-of-care and thus greatly facilitate and improve patient care.

PAD conserves patient blood by using very small blood volumes. Standard clinical laboratories require a minimum of 1 ml of whole blood for small children and 3 ml for adults, but phlebotomists typically draw more for a margin of safety. With repeated testing, such volumes adversely impact small children, particularly those with inborn errors of metabolism who may have frequent hyperammonemia crises in the newborn period. For example, newborn Patient 8 underwent 132 tests during a 31-day hospitalization. This is at least half the blood volume in an average newborn.

The biggest drawback of standard clinical ammonia assays may be venipuncture, which is required to obtain sufficient blood volumes. Venipuncture is particularly problematic for children and neonates, who have veins that are difficult to access and for whom venipuncture can be particularly traumatic. Patient 4 (6 years-old) and Patient 6 (3 years-old) required multiple attempts to gain vascular access by different experienced phlebotomists. The optimal ammonia test should resemble a glucometer test for blood sugar. It should provide immediate results, facilitate repeated testing, and be easy to perform and interpret, ideally by the patients themselves. The second-generation PAD achieved sensitive and accurate measurements from a volume of 10-20 µl deposited on disposable test strips, which is compatible with finger, heel, or earlobe sticks. The simple design of the test strips and low-cost of their components should facilitate high-volume production. Further development requires an analysis of potential environmental effects, including temperature and humidity. Future clinical studies will aim to show equivalence of capillary and venous blood ammonia with actual patients.

Conclusion

We have developed an inexpensive and simple technology utilizing a gas phase ammonia sensor to measure ammonia concentrations rapidly and accurately from small volumes of whole blood. These results provide the basis for development and clinical validation of a device that measures ammonia from capillary blood samples obtained with simple finger, heel, or earlobe sticks at the point-of-care.
Experimental Methods

Enrollment of Healthy Donors and Patients with Hyperammonemia

Collection of blood for this project received approval from the Stanford University Institutional Review Board. To develop the technology, we obtained informed consent from healthy donors. To perform the clinical study of patients with hyperammonemia, we obtained informed consent from the adults and the parents or guardians of the children.

First-generation PAD Construction and Measurement Procedure

The instrument consists of a three-electrode amperometric gas phase ammonia sensor (McNeill International, Inc.) and an alkalization chamber that accepts blood and liberates ammonia. We designed circuitry to accommodate a sensor power supply, a commercial liquid crystal display to show sensor response in real-time, and a 24-bit analog-to-digital converter (PicoTechnology) that transferred current versus time data to a computer.

To avoid the startup cost for injection molding of a disposable alkalization chamber, we purchased a small number of custom reusable glass vials (James Glass Inc.) to test the instrument. Commercially available screw-top lids sealed the vials under a customized rubber septum. Vials containing saturated K$_2$CO$_3$ were stored until use.

To measure blood ammonia, 100 µl of whole blood was injected into a vial and alkalized by vortexing. A cutting tool attached to the sensor housing punctured the septum, creating a gas-tight seal that minimized ammonia escape from the headspace between the blood and the ammonia sensor. To ensure sterilization and reproducibility, and to prevent carryover contamination between uses, vials were soaked in bleach solution, washed with Alconox detergent, steam autoclaved, soaked in saturated potassium carbonate solution, rinsed, and dried in a glassware oven.

Standard curves for the instrument were generated by adding 1 volume of known ammonium chloride standards in water to 99 volumes of fresh heparinized whole blood, fresh plasma, or frozen plasma. Plasma was isolated by centrifugation at 4 °C at 2600 x g for 5 minutes and used fresh or flash-frozen with liquid nitrogen and stored at -80 °C until use. The sensor current was recorded after 100 seconds of equilibration. Standard curves for the instrument response were stable over the course of 12 months.

Limit of Detection (LoD) Estimation

The LoD for the first and second-generation PAD instruments was estimated using a signal-to-noise ratio (SNR) cutoff of 10, the calculated slope of the standard curve (a), and the standard deviation (s) of the baseline current prior to sample introduction:

\[
\text{LoD} = 10 \times (s/a)
\]

Clinical Study Evaluating First-generation PAD versus the Standard Clinical Assay

The clinical study was designed for patients under treatment for hyperammonemia. In the course of patient care, a clinical phlebotomist drew intravenous blood for analysis by the Stanford Clinical Laboratory, following a standard protocol for measuring plasma ammonia levels with the Siemens Dimension EXL. A small volume (typically 0.5 ml) of blood from the same intravenous
draw was heparinized for the clinical study (Figure 3a). A 0.1 ml aliquot was then analyzed by the first-generation PAD.

Second-generation PAD Construction

Design of the second-generation PAD generally followed the first-generation design, except for modifications to accommodate disposable test strips and improve instrument portability. The circuitry was modified for a small rechargeable lithium-ion battery and an on-board battery-charging integrated circuit. The cutting tool of the first-generation PAD was replaced with a chamber machined from PVDF to allow insertion of the test strip into a slot to create a snug fit and seal the system during measurement. Data were collected with a portable data logger (HOBO, Onset Computer Corporation).

Assembly of Disposable Test Strips for Second-generation PAD

Disposable test strips were fabricated from five layers of PVDF film, a piece of nylon mesh, and an Ahlstrom 1281 (Ahlstrom-Muskjo) paper substrate. The PVDF layers were laser-cut to produce 35 mm x 14 mm pieces with circular apertures such that upon assembly the strip retained both a 9.5 mm circular paper substrate and a Triton X-100 coated nylon mesh circle. Individual layers formed a microfluidic channel (0.25 mm deep, 1.6 mm wide) along the strip leading from a sample well to the underside of the coated paper where the nylon mesh was positioned. The nylon mesh provided support for the paper while leaving space for air to be expelled as the blood sample entered the microfluidic channel.

The Ahlstrom 1281 paper (19 mm x 100 mm) was coated in a three-step process. First, the paper was dipped into a 1.5% by weight aqueous solution of Triton X-100, blotted to remove excess liquid, and then dried using forced hot air. Second, the surfactant-coated paper was dipped into a 6 M aqueous solution of K₂CO₃, blotted to remove excess solution, and then placed in a vacuum oven at 85 °C and 200 mTorr for 5 hours. Finally, the carbonate-coated paper was dipped into a 10% by weight of Triton X-114 in methanol, blotted to remove excess liquid, and then dried using forced hot air. The surfactant layers facilitated the rapid and even spreading of the blood across the surface of the paper. After the coating procedure, the paper was cut into 9.5 mm diameter circles for individual test strips. Each test strip was assembled from 5 PVDF layers, a circular nylon mesh, and a paper substrate using an ultrasonic welding machine. Test strips were fabricated in batches and stored under dry nitrogen until use. The low cost of the necessary materials suggest that manufacturing costs will prove to be less than $1 per test strip.

Measurement Procedure for Second-generation PAD

To begin each measurement, a test strip was inserted into the slot of the sensor housing. Approximately 20 µl of the sample was drawn into a Na-heparinized plastic microhematocrit capillary tube (Thermo Fisher Scientific) and then spotted on the sample well, which remains external to the sensor housing after insertion of the strip (Figure 4a). Capillary action draws the sample onto the paper. A surfactant coating on one surface of the channel promotes sample flow onto the coated paper. When the sample contacts the paper, the liquid is wicked both across and into the paper so that the alkalization process begins as sample permeates through the paper. The paper exposes the sample to the detector sensing element on the anode side of the fuel cell. A tight fit between the strip and sensor housing helps seal the analysis chamber. The fuel cell generates a current response that peaks within 45 seconds. For each sample, instrument response was defined as the peak current. To add known amounts of ammonium chloride to whole blood, aqueous
solutions of 10-X concentrated ammonium chloride were prepared and added in a 1:9 volume ratio of aqueous solution to fresh whole blood.

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References


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