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Evaluation of a protein-to-creatinine dipstick diagnostic test for proteinuria screening in selected antenatal care clinics in three Districts in the Bono-East Region of Ghana

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ABSTRACT

Background: Preeclampsia and eclampsia contribute significantly to maternal and newborn deaths worldwide. Early and accurate identification of pregnant women at risk can avert these deaths, but the necessary diagnostics are not widely available. A protein and creatinine ratio, rather than a measurement of protein alone, may provide better identification of proteinuria. The objective of this study was to assess the operational and performance characteristics of the LifeAssay Diagnostics (LAD) Test-it™ protein-to-creatinine ratio (PrCr) urinalysis dipstick test in a representative antenatal care setting (ANC).

Methods: Mixed methods were used to assess the operational and performance characteristics of the PrCr test, including a usability study with 25 participants, a prospective cross-sectional diagnostic accuracy study (N = 1483), and a targeted reassessment of discordant frozen samples (N = 200). Several other commonly used proteinuria tests were included for comparison.

Results: The test demonstrated improved clinical performance for detection of proteinuria over the current standard-of-care tests widely used in Ghana. The LAD PrCr test showed a sensitivity of 50.7% and specificity of 69.2% when run at the point of care. In contrast, the standard-of-care Accu-Tell® protein dipstick test was found to have a sensitivity of 32.4% and a specificity of 82.2%. The LAD test shows minor improvement over the tests currently used in Ghana to detect proteinuria.

Conclusions: The PrCr test offers the potential for improved detection of proteinuria over the standard-of-care tests used in ANC. However, this test and the others evaluated for this study demonstrate limited performance, particularly among samples with a low level of proteinuria. Additional exploration in other clinical use cases, such as triage among high-risk populations, is warranted. The LAD test can also be considered a transition product, as health systems consider adopting next-generation biomarker tests when more readily available.

1. Introduction

Maternal mortality attributed to childbirth or pregnancy-related complications persists as a significant public health challenge globally and is a striking example of inequity in health outcomes for women [1]. An estimated 94 % of these deaths occur in low- and middle-income countries, with 66 % taking place in sub-Saharan Africa [2]. Some of the leading causes of maternal mortality are hypertensive disorders of pregnancy, such as preeclampsia and eclampsia (PE/E), which can lead to seizures and other fatal complications. PE/E accounts for an estimated 16 % of maternal deaths in low-resource settings (LRS) and nearly a quarter of stillbirths and newborn deaths worldwide [3–5]. In LRS, a woman's risk of dying from PE/E is 300 times greater than in a high-resource country [6].

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Currently, assessment of blood pressure and protein in urine (proteinuria) measurements are primary clinical indicators for identifying the risk of developing PE/E in pregnant women. Elevated blood pressure and total proteinuria can be considered significant and, when combined, warrant a diagnosis of preeclampsia [7–9]. Danger signs of preeclampsia, including frontal headaches, edema, and blurred vision, may also be used to support diagnosis. Early assessment of risk can facilitate early intervention and management of the condition, prior to the development of complications that pose a risk to the health of mothers and babies. Global recommendations indicate a pregnant woman should have at least four antenatal care (ANC) visits over the course of pregnancy, with blood pressure measurement and proteinuria measurement assessed during each visit. However, some women do not attend ANC regularly or the diagnostic tools needed for these assessments are not readily available. This can lead to difficulties or delays in the detection of complications, including PE/E [10,11].

There also are barriers to obtaining accurate proteinuria measurement with the existing tools widely available in LRS. The gold standard for measuring proteinuria is 24-hour urine collection [12–14]. However, it is inconvenient given the time and resources required and is only available in some secondary- and tertiary-level settings due to its high cost and complexity. In addition to these barriers to use, 24-hour urine collection is subject to errors, such as incomplete sample collection that renders the result inaccurate [14–16]. In LRS, protein measurement using a urine dipstick test is the most common method for proteinuria assessment due in part to its low complexity and cost [17]. However, the clinical utility of protein-only dipstick measurements is limited, and the World Health Organization considers the accuracy of these tests to be low [10]. A recent systematic review found that the accuracy of protein-only dipstick measurements ranges widely, with performance as low as 20 %–40 % sensitivity and specificity [18]. Other studies have shown negative and positive predictive values for protein-only dipsticks at 34 % and 36 %, respectively, and questioned their utility in accurately determining significant proteinuria [19]. One of the causes of poor test performance is that protein-only dipsticks cannot adjust for daily fluctuation in body hydration. This can lead to both over- and underestimation of protein measurements, depending on the level of urine dilution [20].

Due to the limitations of protein-only urine dipsticks and 24-hour urine collection, some health guidelines, such as those from the UK National Institute for Health and Care Excellence, have recommended the use of the protein-to-creatinine ratio (PrCr) or the albumin-to-creatinine ratio for detection of proteinuria [7]. When using a chemistry analyzer, the most widely reported threshold for differentiating an abnormal versus normal proteinuria result is a PrCr of greater than or equal to 0.3. Additionally, in the World Health Organization 2017 updated guidelines to *Managing Complications in Pregnancy and Childbirth*, a urinary PrCr of 0.3 or greater was added to the diagnostic criteria for proteinuria, alongside 24-hour urine collection, rather than replacing it [8].

The measurement of the protein and creatinine concentrations in a spot urine sample is determined and calculated in laboratory settings using chemistry analysis [17]. However, like other methods that rely on complex medical infrastructure, Brown et al. warn that "as with 24-hour urine collection, this laboratory-based testing method is similarly inaccessible in LRS due to cost, complexity, and infrastructure requirements. Further, this method also relies on effective referral networks" [21]. As such, there is a critical gap in accessible, low-cost, and accurate screening tools for proteinuria at the point of care. Specifically, point-of-care measurement of protein and creatinine concentrations can confer the benefits of both the improved accuracy of the PrCr and the easy-to-use format of a urine dipstick.

2. Study aim and rationale

Early development of a prototype low-cost urine dipstick test for point-of-care PrCr measurement, the Test-it™ PrCr urinalysis dipstick test (Test-it is a trademark of LifeAssay Diagnostics [Pty] ltd, Cape Town, South Africa), was advanced through laboratory-based verification in 2015 (Fig. 1). This test works to improve accuracy of the proteinuria determination over protein-only dipsticks by normalizing the protein measurement with the creatinine measurement to account for a patient's level of hydration. Results can be obtained in 60 s using a visually read colorimetric scale that supports clinical decision-making and indicates whether the PrCr is considered normal or abnormal, based on the manufacturer's recommended PrCr threshold for abnormal proteinuria (=0.30). It is produced in a manufacturing facility with quality systems accredited to international standards (International Organization for Standardization [ISO] 9001 and ISO 13485), and the test is CE (Conformité Européene) marked at the global level with registration underway in countries across Asia and Africa. The instructions for use are available in Appendix A.

Preliminary results from laboratory-based verification in the United States using characterized urine samples demonstrated the Test-it PrCr urinalysis dipstick had 85 % sensitivity (95 % CI, 75.3 %–91.6 %) and 71 % specificity (95 % CI, 56.9 %–82.9 %) for correct disease classification compared to PrCr reference measurement using a laboratory-based chemistry analyzer [22].

While results from early laboratory verification in the United States indicated a promising performance for the Test-it PrCr urinalysis test, critical evidence was needed to verify its performance and usability within targeted ANC settings to determine its readiness for deployment. The purpose of the current study was not only to provide evidence on the test performance using a larger, representative sampling but also to clarify considerations and resources for optimal use of the PrCr test during future implementation in ANC in LRS.

The overall objective of the current study was to assess the operational and performance characteristics of the PrCr test in Ghana. In 2016, user research was conducted to evaluate the usability of the LifeAssay PrCr test, with a focus on test interpretation with a prototype

Fig. 1. Test-it protein-to-creatinine ratio (PrCr) urinalysis dipstick test. * Test-it is a trademark of LifeAssay Diagnostics (Pty) ltd.

job aid, device failures, and general end-user feedback. In 2018, a diagnostic evaluation was conducted at the same sites to assess the accuracy of the PrCr dipstick test as compared to a laboratory-based reference assay and the current standard of care for detection of proteinuria. Both studies were conducted in the Bono-East Region of Ghana and within representative settings where proteinuria testing is performed to support routine ANC.

3. Methods

3.1. Study setting and population

The study was conducted in the Techiman, Kintampo North, and Kintampo South Districts of the Bono-East Region of Ghana, in what was formerly known as the Brong-Ahafo Region, between 2016 and 2020 Fig 2. This area has experienced a steady decline in maternal mortality over the past ten years [23]. In this region, ANC attendance is high, with 98.8 % of women receiving care from a skilled provider (a doctor, nurse, nurse/midwife, or community health officer) and 90.3 % of women attending at least four ANC visits. Around 80 % of women in this region deliver in facilities attended by a skilled health care provider [8].

Health services in the study area are delivered at three levels: the first comprises the Community Health Planning Services system, which provides routine ANC at the community level; the second includes health centers, private maternity homes, and private clinics, which have trained midwives and other health care professionals who provide ANC and delivery services but not surgical procedures; and the third and highest includes the district hospitals (Kintampo Municipal Hospital and Kintampo South District Hospital), where all ANC services, including delivery and surgery, are provided by doctors and other health care professionals. All three levels of service delivery were included in the study.

3.2. Study design

Mixed methods were used to assess the operational and performance characteristics of the PrCr test (Fig. 3). First, in 2016, a usability study was conducted with ANC staff at 11 health facilities in Kintampo North and South Districts. Second, a large prospective cross-sectional diagnostic accuracy study was initiated in 2018 in the same facilities. Finally, a targeted subset of frozen samples was retested in 2020.

For the usability study, a purposive sample of 25 health workers with varying roles were enrolled and trained on the use of the test: midwives (N = 8), community health nurses/officers (N = 10), and nurses or nurse assistants (N = 7). Study staff observed as the usability participants ran the test using control samples and interpreted the results. Observations were recorded using a structured guide and checklist with key steps. Participants also provided feedback on the test and interpretation tools using a semi-structured questionnaire.

A cross-sectional diagnostic accuracy evaluation was also conducted at outpatient ANC clinics. The primary aim of the study was to assess the clinical performance of the LifeAssay Diagnostics (LAD) Test-it PrCr test against a chemistry analyzer as a laboratory-based reference standard for PrCr measurement. Based on the expected prevalence of proteinuria at the study sites (7 %) with a confidence interval of 90 %, and a 10 % maximum margin of error, the study aimed to recruit 3,300 pregnant women with a minimum of approximately 200 women with proteinuria [24–26]. Multiple comparisons across protein and creatinine measurement platforms were conducted.

At multiple ANC clinics, pregnant women over the age of 18 and in the last trimester of their pregnancies were recruited and consented into the study. Study participants provided a urine sample as per routine care and study staff tested the sample using the LAD Test-it PrCr test and the Accu-Tell® Urine Reagent Strip (Accu-Tell is a registered trademark of AccuBioTech), a protein-only urine dipstick test currently registered and routinely used in Ghana. Both tests were conducted at the point of care on fresh urine samples immediately after collection. The test result was recorded and classified as either abnormal or normal for proteinuria based on the test instructions for use. Patient samples were transferred to the Kintampo Health Research Centre laboratory and tested using a chemistry analyzer and both dipsticks, and then frozen. The results of the chemistry analyzer were generated by measuring the ratio of protein and creatinine concentrations in mg/dL and applying a threshold of 0.30 to classify results as abnormal or normal for proteinuria.

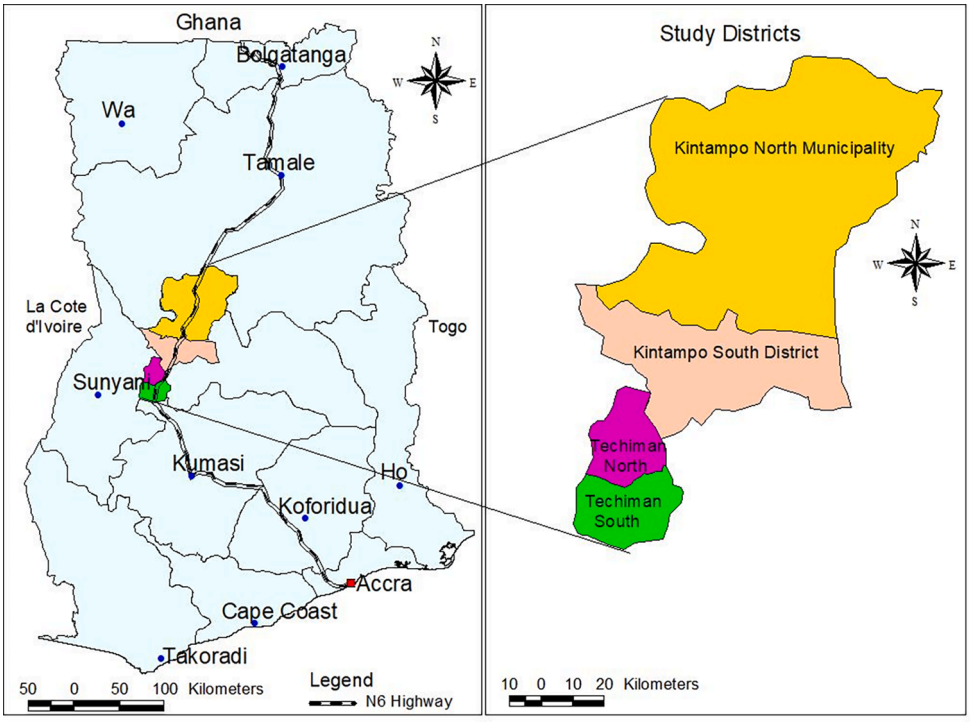

Fig. 2. Map of study area.

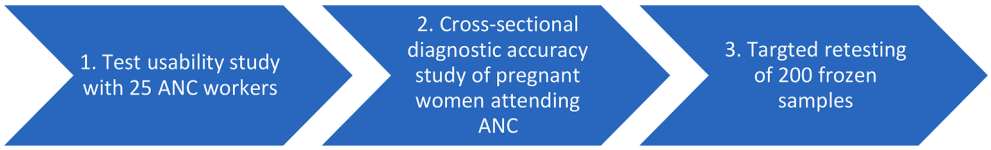

Fig. 3. Multiphase mixed-methods diagnostic evaluation of operational and performance characteristics.

An interim analysis was conducted after the enrollment of 1483 participants from September 2018 to March 2019. The interim analysis showed a greater than expected discrepancy between the PrCr test performance in the field as compared to the prior laboratory verification studies. Additional testing of discordant samples was pursued to understand the difference in observed test performance between initial laboratory verification and this field validation, and further enrollment was halted.

3.3. Sample retesting

In February and March 2020, a subset of 200 frozen urine samples were retested. Samples were selected to include discordant samples, all women with hypertension, and a random selection across both protein and creatinine dynamic ranges. The retesting included the test used in the initial prospective sample test (LifeAssay Diagnostics PrCr test [LAD], Accu-Tell Urine Reagent Strip, and reference chemistry analyzer), as well as three additional point-of-care tests: Uristix® (Uristix is a registered trademark of Siemens), Mission® Urinalysis Strips (Mission is a registered trademark of ACON Laboratories), and Siemens Diagnostics Multistix PRO 10 LS Urinalysis Strips. All comparator tests relied on the visual interpretation of a colorimetric chart to classify normal and abnormal results. See outline of all study assays in Table 1.

Table 1
All study assays.

Test name	Rationale for inclusion in study	Parameters measured	Samples tested
LifeAssay Diagnostics (LAD) Test-it™ PrCr test	Investigational product	PrCr	• Fresh samples from large prospective cohort at the point of care and in lab Frozen samples from the targeted retesting in lab
Accu-Tell® Urine Reagent Strip (registered trademark of AccuBioTech)	Point-of-care comparator	Protein only	• Fresh samples from large prospective cohort at the point of care and in lab Frozen samples from the targeted retesting in lab
Uristix® urinalysis test strips (registered trademark of Siemens)	Point-of-care comparator	Protein only	Frozen samples from the targeted retesting in lab
Mission® Urinalysis Strips (registered trademark of ACON Laboratories)	Point-of-care comparator	Protein only	Frozen samples from the targeted retesting in lab
Siemens Diagnostics Multistix PRO	Point-of-care comparator	PrCr	Frozen samples from the targeted retesting in lab
Chemistry analyzer	Reference test	PrCr	• Fresh samples from large prospective cohort in lab only Frozen samples from the targeted retesting in lab

The Siemens Multistix Pro 10 LS was included in the study to serve as a comparator: a commercially available point-of-care test that offers both protein and creatinine measurement using a similar urinalysis dipstick format but with a high cost that precludes widespread use in LRS. The Accu-Tell, Uristix, and Mission dipsticks were included because they are commonly used products for protein measurement in ANC in Ghana. The same chemistry analyzer was included as the laboratory-based reference standard for spot urine measurement of protein and creatinine.

Sample retesting took place in the Kintampo Health Research Centre laboratory. Samples were thawed to room temperature and then retested using the urinary dipsticks and then an aliquot on the chemistry analyzer as the reference method. The primary aims of the retesting were to assess the clinical performance of the LAD Test-it PrCr test against the chemistry analyzer, as the laboratory-based reference standard, and against the Siemen's Multistix Pro 10 LS, as a point-of-care comparator.

3.4. Statistical methods

Results from the usability observation checklists were tabulated in Excel. For the diagnostic accuracy study, statistical analyses were conducted in Stata 13.1 (StataCorp, College Station, TX). The distribution of the following characteristics was evaluated: age, facility, hypertension status, gestational age, participant-reported symptoms and care, and previous labor complications. Multiple analyses of the data from the complete cohort were conducted. LifeAssay Diagnostics (LAD) PrCr and Accu-Tell dipstick test results read both at the point of care and in the lab were classified as either normal or abnormal, according to manufacturers' instructions. The diagnostic performance (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) of the LAD PrCr test in the field and lab and the Accu-Tell dipstick compared to the reference chemistry analyzer was determined using the 0.30 PrCr quantitative threshold.

A PrCr 0.30 threshold was applied to the lab reference chemical analyzer test for both the original and sample retest study. Individuals with a PrCr of less than 0.30 were considered normal, whereas those with a PrCr of greater than 0.30 were considered abnormal. Similarly, a PrCr 0.30 threshold was applied to the LAD test in the original study to classify individuals as normal or abnormal, as per manufacturers' recommendations in the instructions for use. However, the Siemen Multistick PRO manufacturer recommends a PrCr threshold of 0.15 to classify individuals as normal or abnormal. As such, this threshold was also applied to both the LAD PrCr test and the Siemen Multistick PRO test in the sample retest study.

In addition, receiver operating characteristic (ROC) curves comparing the index tests and reference assay were plotted at the 0.30 threshold and the area under the curve (AUC) was calculated. As protein-to-creatinine ratios are measured on an ordinal scale, it is useful to assess performance of the diagnostic test over the range of possible thresholds for the predictor variable. This is achieved by an ROC curve that includes all the possible decision thresholds for a diagnostic test result [27]. The AUC is an effective way to summarize overall diagnostic performance.

3.5. Ethics

Ethical approvals for the usability study, performance validation study, and laboratory-based retesting were received from the Ghana Health Service Ethical Review Committee, the Kintampo Health Research Centre Institutional Ethics Committee, the PATH Research Ethics Committee, and the Ghana Food and Drugs Authority. Written informed consent was obtained from all study participants. Only registered tests were used to inform patient management at the point of care. Results of the reference assay that suggested a clinically relevant level of elevated proteinuria were reported back to the participant's health care provider at the facility. The study team ensured participant's confidentiality was maintained. Personal identifiers were not included in study reports. The informed consent procedure was conducted in a private setting. Study data were recorded into an electronic study database. All electronic data were kept in a password-protected server in a secure location and remained separate from participant identification information to ensure confidentiality.

4. Results

4.1. Usability testing

Health workers were generally familiar with the strip concept and found it to be simple, easy to use, and satisfactory. They appreciated that the test was fast and noted that that interpretation chart was useful, though they were not adept at using the grid style interpretation chart (see Appendix A for the interpretation chart). All participants said they would want to use this test, particularly if it performs better than current tests. Most participants felt confident in using the test and felt like using the test would bring some benefit to ANC. As currently multiple different brands of dipstick tests are used across clinics in Ghana, using the LAD PrCr test was seen as an opportunity for greater standardization of test interpretation within and between clinics. Users were familiar with a dipstick that included a protein pad and a glucose pad, both of which were interpreted independently. Participants noted that if this test were used in routine care, a second test would be needed to screen for glucose. Recommendations for improvement of the test include some refinement to the language in the instructions for use and modification of the color of the creatinine marker to match the test and the instructions.

Table 2 summarizes observations from the usability participant observation, for each step of the tests. Critical user failures centered on the matching of the color on the creatinine pad to the colors in the instructions. Another common challenge was the concept of the ratio interpretation.

Table 2
Steps involved in the test workflow and the associated user observations at each step.

Step	Observations
1. Put on gloves	Participants will use gloves if available but may ration them; health workers did not consider contamination a significant concern when using dipsticks.
2. Read instructions for use	• Many participants (12/25) incorrectly used instructions for their current test, particularly regarding timing (i.e., dip for 3 s and read after 3 min). Most participants (22/25) read instructions not before testing but after dipping strip, while waiting for 1 min. Three participants noted expiry date.
3. Remove test strip	No difficulties.
4. Open sample container	Participants ask women to open their own containers.
5. Dip stick for 1 s	• Most participants (21/25) kept stick in urine for longer than 1 s (7–10 s). Two participants did not dip strip far enough to cover both pads.
6. Blot on absorbent paper	• Only one participant blotted. Discussion suggested that "blot" is not a well understood word.
7. Wait for 1 min	• Three participants read test straightaway. About half of participants (13/25) used a timing device (cell phone or breast watch).
8. Read at 1 min	• To read, participants generally held strip in dominant hand, vertical to canister in other hand. Rotated canister or moved strip to match. Participants did not touch strip to canister; no issues with sample spillage or contamination despite not blotting strip.
9. Matching color of test to color on canister	• Four participants took several minutes (4–5 min) to select color; most made choice within 1 min. Three participants changed their decisions about color while reading. Protein was matched correctly by 23/25 participants. Creatinine was matched correctly by 19/25 participants. Six participants mentioned that the Cr was difficult to match.
10. Interpretation of PrCr ratio	• The ratio concept was not well understood. Grid interpretation chart had to be taught and was not intuitive. Some participants (5/25) ignored the Pr, provided interpretation of Pr and Cr independently, or referred to Cr as glucose.

4.2. Performance evaluation

The following results are from the original cross-sectional prospective data collection. Table 3 below summarizes the participant demographics of the study cohort, N = 1483 women in their third trimester of pregnancy. The study population was largely healthy, with about 25 % classified as pre-hypertensive or hypertensive and only five women with a medical history of preeclampsia or eclampsia. Fewer than 10 % of all women reported any of the clinical symptoms associated with preeclampsia or eclampsia.

Performance characteristics of the LAD PrCr test run in the laboratory and at the point of care, as well as the Accu-Tell test run at the point of care, including specificity, sensitivity, and positive and negative predictive values are shown below in Table 4 for the full study cohort.

The LAD PrCr test showed a sensitivity of 50.7 % when run at the point of care, as compared to 41.2 % when run in the laboratory. Specificity was higher at 69.2 % and 83.2 % at the point of care and in the laboratory, respectively. In contrast, the standard-of-care Accu-Tell protein dipstick test, which was run only at the point of care, had a sensitivity of 32.4 % and a specificity of 82.2 %.

As described above, a targeted subset of samples was selected for retesting. The results of this retesting are presented in Table 5, below.

Good specificity was observed across all tests but sensitivity was low. Using the 0.3 threshold for abnormal versus normal, the LAD PrCr test demonstrated increased sensitivity over all urine dipsticks used in the study. Similar sensitivity was observed for the LAD PrCr test in the retesting as in the lab-based testing during the original study, although specificity was improved.

4.3. ROC curves and discussion of alternate thresholds

At the 0.30 threshold, the AUC values across the urine dipstick tests were comparable (Table 5). The LAD PrCr test had the strongest performance with an AUC of 0.68, followed by the Siemens (0.65), Mission (0.63), and Accu-Tell (0.62) tests. There is general consensus that an AUC of 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is excellent, and greater than 0.9 is outstanding [27]. It is notable that all of the dipsticks at the 0.30 threshold fall below the 0.7 AUC threshold for acceptable performance.

As discussed, alternative thresholds for the PrCr have also been explored. A threshold of 0.15 is recommended by the manufacturer of

Table 3
Participant demographics (N = 1483).

Average age, in years (SE)	28.3 (6.1)
Reference lab chemistry analyzer PrCr	
less than 0.30, # (%)	1204 (81.2)
≥ 0.30, # (%)	279 (18.8)
Hypertension status	
Normal, # (%)	1102 (74.3)
Pre-hypertension, # (%)	341 (23.0)
Hypertension, # (%)	40 (2.7)
No data, #	0
Gestational age, in weeks	
Mean (SE)	32.5 (4.6)
Median (IQR)	33.0 (29–36)
No data, #	2
# (%) per facility	
Kintampo Municipal Hospital	608 (41.00)
Kintampo South District Hospital, Jema	86 (5.80)
Holy Family Hospital, Techiman	674 (45.45)
Glory Prince of Peace Maternity Home, Kintampo	94 (6.34)
Other	21 (1.47)
Reported symptoms and care, # (%) responding "yes"	
Dizziness	27 (1.82)
Severe headache	65 (4.38)
Unusual swelling	3 (0.20)
Abdominal pain	134 (9.04)
History of hypertension	15 (1.01)
# with previous labor complications	
Preeclampsia	3
Eclampsia	2
Excessive bleeding	29
Postpartum hemorrhage	2

Abbreviations: IQR, interquartile range; PrCr, protein-to-creatinine ratio; SE, standard error.

Table 4
Clinical performance for detection of proteinuria as compared to a reference chemistry analyzer Pr:Cr result of greater than 0.30 for classification of proteinuria (N = 1483).

	LAD PrCr test (Lab)	LAD PrCr test (POC)	Accu-Tell Protein (POC, standard of care)
Total number	1482*	1477*	1477*
True positives	115	141	90
True negative	1001	828	986
False positives	202	371	213
False negatives	164	137	188
Sensitivity % (95 % CI)	41.2 (35.4–47.2)	50.7 (44.7–56.7)	32.4 (26.9–38.2)
Specificity % (95 % CI)	83.2 (81.0–85.2)	69.1 (66.4–71.7)	82.2 (80.0–84.4)
PPV% (95 % CI)	36.3 (31.0–41.8)	27.5 (23.7–31.6)	84.0 (81.8–86.0)
NPV% (95 % CI)	85.9 (83.8–98.9)	85.8 (83.4–87.9)	84.0 (81.8–86.0)
AUC (95 % CI)	0.62 (0.59–0.65)	0.60 (0.57–0.63)	0.57 (0.54–0.60)

Abbreviations: AUC, area under the curve; CI, confidence interval; LAD, LifeAssay Diagnostics; NPV, negative predictive value; POC, point of care; PPV, positive predictive value; PrCr, protein-to-creatinine ratio.
*The analytical population here excludes one participant for which no data was available and five additional participants whose samples were tested only in the lab and not at the point of care.

the Siemens Multistick PRO for the classification of normal or abnormal samples. Applying a 0.15 threshold to the data generated in the sample retesting, the Siemens Multistix PRO provided the best overall performance for detection of proteinuria (Table 6). The sensitivity of the LAD PrCr test improved as well, increasing from 41.9 % to 45.2 %. The AUC values increased for both tests. The AUC for the Siemens dipstick at the 0.15 cutoff exceeded the 0.7 threshold to be considered "acceptable" performance, and the LAD PrCr test nearly attains it (0.69). Notably, the Siemens dipstick test is not available or registered in Ghana and thus of the tests available in this setting, the LAD test obtained the highest performance.

Table 5
Clinical performance for detection of proteinuria as compared to a reference chemistry analyzer PrCr result of greater than 0.30 for classification of proteinuria in the sample retest study (N = 200).

	LAD PrCr	Siemens PrCr	Accu-Tell	Mission
Total number	200	196**	200	200
True positives	26	22	17	17
True negative	131	127	132	135
False positives	7	8	6	3
False negatives	36	39	45	45
Sensitivity % (95 % CI)	41.9 (29.5–55.2)	36.1 (24.2–49.4)	27.4 (16.9–40.2)	27.4 (16.9–40.2)
Specificity % (95 % CI)	94.9 (89.8–97.9)	94.1 (88.7–97.4)	95.7 (90.8–98.4)	97.8 (93.8–99.5)
PPV% (95 % CI)	78.8 (61.1–91.0)	73.3 (54.1–87.7)	73.9 (51.6–89.8)	85.0 (62.1–96.8)
NPV% (95 % CI)	78.4 (71.4–84.4)	76.5 (69.3–82.7)	74.6 (67.5–80.8	75.0 (68.0–81.1)
AUC (95 % CI)	0.68 (0.62–0.75)	0.65 (0.58–0.72)	0.62 (0.56–0.67)	0.63 (0.57–0.68)

Abbreviations: AUC, area under the curve; CI, confidence interval; LAD, LifeAssay Diagnostics; NPV, negative predictive value; PPV, positive predictive value; PrCr, protein-to-creatinine ratio.
**The analytical population here excludes four participants whose samples were not tested using the Siemens test.

Table 6
Clinical performance for detection of proteinuria as compared to reference chemistry analyzer PrCr based on 0.15 threshold for classification of proteinuria.

	LAD PrCr	**Siemens PrCr**
Total number	200	196***
True positives	28	41
True negative	128	114
False positives	10	21
False negatives	34	20
Sensitivity % (95 % CI)	45.2 (32.5–58.3)	67.2 (54.0–78.7)
Specificity % (95 % CI)	92.8 (87.1–96.5)	84.4 (77.2–90.1)
PPV % (95 % CI)	73.7 (56.9–86.6)	66.1 (53.0–77.7)
NPV % (95 % CI)	79.0 (71.9–85.0)	85.1 (77.9–90.6)
AUC (95 % CI)	0.69 (0.62–0.76)	0.76 (0.69–0.83)

Abbreviations: AUC, area under the curve; CI, confidence interval; LAD, LifeAssay Diagnostics; NPV, negative predictive value; PPV, positive predictive value; PrCr, protein-to-creatinine ratio.
***The analytical population here excludes four participants whose samples were not tested using the Siemens test.

5. Discussion

5.1. Usability testing

The usability data generated suggest that the LAD PrCr test would be well accepted by ANC staff in representative settings of use. The critical user errors, primarily focused on color matching, are shared by all visually interpreted urine dipstick tests. Some minor user errors can be remedied with improved operational guidance and training materials. Importantly, the results of the usability test indicated that innovative interpretation charts are needed to move users from the reading and interpretation of a single parameter, such as protein or glucose separately, to the reading of two parameters—protein and creatinine, and the interpretation as a ratio. After the usability study, various

interpretation charts were developed and vetted with users. The final design was incorporated into the instructions for use in the current LAD product (https://www.lifeassay.com/products_urine_analysis.php).

Qualitative feedback from users also revealed that the use of multiple different protein dipsticks across ANC clinics is common, and that the lack of standards for dipstick test use and interpretation may impact their effective use. Finally, the usability data indicated that an understanding of the context of test introduction will be key, as other parameters, such as glucose in the case of Ghanaian clinical guidelines and practices, will be important and may need to be integrated into the same strip.

5.2. Performance testing

Overall, the PrCr test demonstrated some improved clinical performance for detection of proteinuria over the current standard-of-care tests widely used in Ghana, the Mission and the Accu-Tell dipsticks. This incremental improvement offers a 10 %–15 % improvement in sensitivity over other tests. The LAD PrCr test demonstrated comparable performance to the Siemens Multistix PRO 10 LS, a more expensive test that is also approved by stringent regulatory authorities but not available for use in Ghana.

An important consideration in the interpretation of the performance evaluation is that the study was performed in routine ANC settings with a healthy cohort of pregnant women. As such, only 19 % of the women met the clinical definition of abnormal proteinuria, with most women having only less severe proteinuria based on reference PrCr values at or near the threshold of 0.3 for abnormal classification.

Among the larger cohort of women (N = 1483), the high degree of variation between the lab and point-of-care test results indicated that variation between users is a key consideration for this test. This high degree of variation was likely a factor in the degradation of test performance observed between the early verification data generated in a controlled laboratory setting and these studies conducted in Ghana. However, the sample retesting resulted in similar results to that of the larger cross-sectional study. This suggests that there may be challenges in standardizing approaches for tests with colorimetric visual interpretation. The LAD test needs to be read at 60 s, which can be difficult both in point-of-care and laboratory settings. There have been some efforts to automate this interpretation by test readers and mobile applications, but these interpretation tools are not yet widely available and risk a significant increase in the costs associated with urine dipsticks.

The retesting offered an opportunity to compare multiple commonly used point-of-care tests, many of which are registered for use in Ghana. The sensitivities observed, particularly among samples with low proteinuria, were lower than those reported in product labels. This finding is in line with guidance from the World Health Organization indicating that currently available proteinuria dipsticks have low accuracy. Proteinuria tests may offer improved performance among specific at-risk populations. Subsequent evaluation of the PrCr test should be conducted within a population with higher rates of preeclampsia and hypertension, such as through targeted testing of at-risk pregnant women. These evaluations should also take into consideration the need for clear operational guidance for the use of the test.

Biomarker-based tests have great potential in allowing for more accurate and potentially earlier identification of high-risk women. The lack of available commercial biomarker-based test products, particularly in affordable and field-friendly formats, greatly limits their use in the care of pregnant women in low- and middle-income countries [28]. One such test uses urinary adipsin, a recently described diagnostic biomarker for PE/E with strong clinical performance previously reported (sensitivity and specificity up to 99 % and 93 %) and with compatibility for use in low-cost lateral flow–based rapid diagnostic test format [29,30]. As research and development for the next-generation biomarker tests continue, the currently available, registered, and accessible LAD test can be considered a transition product. Expanded use of the PrCr test may spur some of the health system adaptions and transitions required for transition to the effective use of biomarker-based tests.

6. Conclusions

The PrCr test offers the potential for minimal improved detection of proteinuria over the standard-of-care tests currently used in ANC. However, the LAD and other Pr and PrCr tests in this study (Accu-Tell Urine Reagent Strip, Uristix urinalysis test strips, Mission Urinalysis Strips, and Siemens Diagnostics Multistix PRO) demonstrate limited performance, particularly among samples with low levels of proteinuria. User interpretation remains a key potential source of error that is common across all urine dipstick tests. Given that simple and low-cost urine dipsticks remain important and necessary tools for screening and diagnosing PE/E in low- and middle-income countries, clear training and implementation guidance should focus on best practices identified for results interpretation. Additional research on use of the PrCr test in different clinical use cases would further clarify the utility of PrCr tests. Finally, better use of these available tests can be considered a transitional step toward the introduction of accurate and easy-to-use biomarker-based tests for PE/E.

Declarations

Ethics approval and consent to participate

Ethical approvals for the usability study, performance validation study, and laboratory-based retesting were received from the Ghana Health Service Ethical Review Committee, the Kintampo Health Research Centre Institutional Ethics Committee, the PATH Research Ethics Committee, and the Ghana Food and Drugs Authority. Written informed consent was obtained from all participants.

Consent for publication

Our manuscript does not contain any individual person's data in any form.

Availability of data and material

The data that support the findings of this study are available from the corresponding author, [EGG], upon reasonable request.

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Authors' contributions

EGG is responsible for the funding acquisition, study conceptualization, and original draft of the manuscript. DAG is responsible for the study methodology, investigation, and manuscript review and editing as well as project supervision. CTA is responsible for the funding acquisition, investigation, manuscript review and editing, as well as project supervision. PB is responsible for the analysis and manuscript review and editing. RB is responsible for the investigation and manuscript review and editing. SK is responsible for project administration and manuscript review and editing. SN is responsible for project supervision and manuscript review and editing. JR is responsible for conceptualization and manuscript review and editing. BTL is responsible for project conceptualization, methodology, analysis, funding acquisition, and manuscript review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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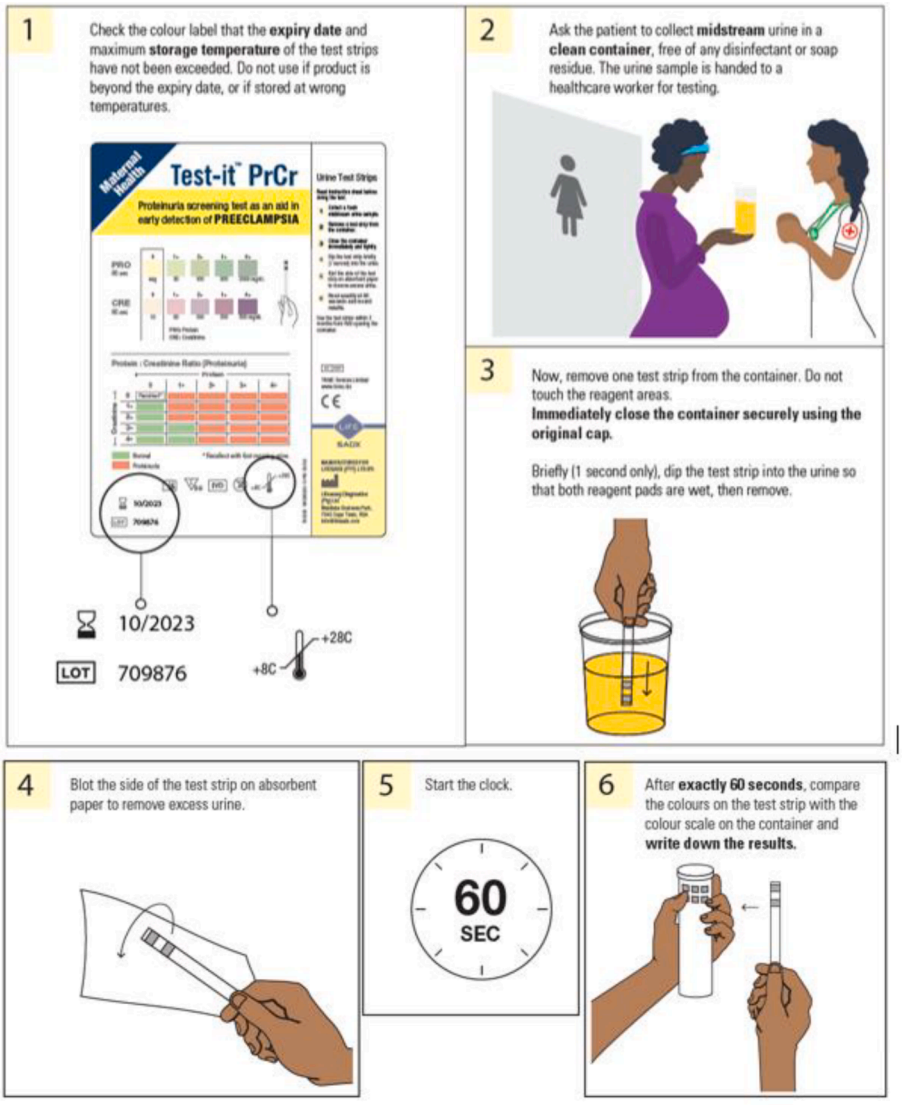
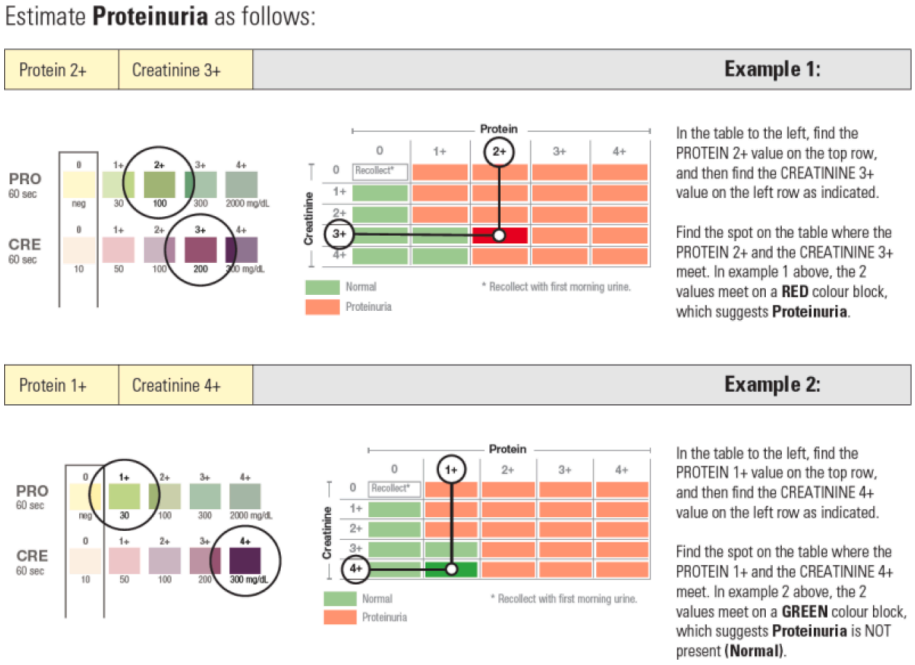
Appendix A. LifeAssay Diagnostics (LAD) Test-it™ PrCr urinalysis dipstick test Instructions for use





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