Proteinuria measurement and urine-based markers for preeclampsia diagnosis

Technology Opportunity Assessment

Prepared for the Merck for Mothers Program
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Statement of need

Preeclampsia (PE) is a life-threatening disorder that only occurs during pregnancy, childbirth, and the postpartum period and is characterized by high blood pressure (hypertension) and protein in the urine (proteinuria). Convulsions (fits) with signs of PE indicate eclampsia, although occasionally convulsions occur in the absence of hypertension with proteinuria. Preeclampsia and eclampsia (PE/E) are among the leading causes of maternal death and disability worldwide. The World Health Organization (WHO) estimates that PE/E accounts for at least 16% of maternal deaths in settings with limited resources that lack the skilled providers and facilities required for prevention, identification, and management of the condition.1 In most countries, PE/E rank second only to hemorrhage as specific, direct causes of maternal death. The risk of PE/E varies greatly depending on where a woman lives; the risk that a woman in a low-resource country will die of PE/E is approximately 300 times greater than that for a woman in a high-resource country.2

Positive maternal and perinatal outcomes for women with PE/E depend on how soon the condition is identified and how quickly the woman has access to the treatment package.* Prevention for PE/E has focused on antenatal screening for high blood pressure and proteinuria as part of focused antenatal care (ANC).† The presence of proteinuria (total protein excretion exceeding 300 mg/24 hours)3 changes the diagnosis from gestational hypertension to PE, and detection of proteinuria is key for making a diagnosis of PE. In practice, however, the number of women accessing ANC remains far below recommended visitation levels. ANC utilization is around 68% in low- and middle-income countries compared to 98% in high-resource settings.4 In fact more than half of all births occur at home, and many pregnant women receive no prenatal care.5 Furthermore, the urgency to quickly get treatment is even more acute for pregnant women in rural and even peri-urban areas who do not have easy access to well-equipped clinics with trained staff. In areas with little or no prenatal care, case fatality rates are close to 3,000 per 10,000 births (30%), and most of the deaths appear to occur among “un-booked” women—women who were not seen for any pregnancy-related medical care until the first seizure. These women, if they receive any care at all, are often brought to a hospital in a coma after multiple seizures, and approximately 30% to 50% or more of these women die.5

The “gold standard” for measuring proteinuria requires a 24-hour urine collection to directly determine the daily total protein or albumin excretion. Such collections (typically during a minimum two-night hospital stay) are resource intensive and require refrigeration of the urine during collection and

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* The treatment package includes in-patient monitoring, anticonvulsive and antihypertensive therapy, optimal timing of childbirth, and skilled attendance at birth.
† Focused ANC is evidence-based, goal-directed care that is tailored to the gestational age of pregnancy and individualized to each woman. It emphasizes quality of visits over quantity of visits and is conducted by a skilled health care provider. Goals of focused ANC include early detection and treatment of complications, prevention of problems, birth preparedness/complication readiness, and the promotion of healthy practices to help ensure a positive health outcome for the woman and her baby. Focused ANC is provided through a women-centered approach that values the dignity and value of each woman and her family.
laboratory-based analysis. In fact, the number of 24-hour collections considered incomplete during studies of pregnant women approaches 50%, more than double those in studies of nonpregnant populations.\(^6\) Further complicating this procedure is the fact that the laboratory-based assays require trained staff in a laboratory, typically a central laboratory at a major hospital. As a result, timed collections are rarely practical if greater reach is sought outside formal clinics and urban settings.

There is, therefore, a great need for low-cost devices to measure proteinuria that meet or approach WHO’s ASSURED criteria\(^7\) (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users). Like protein-only dipsticks, proteinuria screening devices must remain affordable, widely available, and functional at the most peripheral points of care. To impact morbidity and mortality in PE/E, the urinalysis device should not only improve accuracy of proteinuria measurements but should also increase reach to the community level by simplifying, to the greatest extent possible, how proteinuria is measured. Increased access to tests that meet WHO’s ASSURED criteria and are affordable would greatly improve the identification of women with PE and increase their opportunity to access treatment packages, ultimately increasing the likelihood of survival for mothers with PE and their infants.

**Technology solutions landscape**

This landscape analysis covers biomarkers and methods for their determination that are candidates for PE screening. These methods are split into those designed for the determination of proteinuria (total protein or albumin determination—see Section I) and those for the determination of urine biomarkers that are more specifically correlated with PE (see Section II).

Although the measurement of proteinuria is part of the formal diagnostic criteria of PE, it is by itself a less than perfect predictor of either maternal or fetal complications in women with PE.\(^8\) Studies have shown that 10% of women with clinical and/or histological manifestations of PE have no proteinuria, and 20% of women with eclampsia do not have significant proteinuria prior to their seizure.\(^9,10\) To further complicate the diagnosis of PE based on proteinuria, primary renal disease and renal disease secondary to systemic disorders, such as diabetes or chronic hypertension, are also usually characterized by proteinuria and may first present in pregnancy. In addition, each of the current methods for proteinuria screening possesses shortfalls which make use in low-resource settings (LRS) challenging. These challenges are detailed in Appendix A which delineates a total of 16 technologies, the majority of which are commercially available and a few of which are in development or in the conceptual stage. All 16 technologies are grouped by assay categories for comparative purposes. Each technology varies in accuracy, cost, simplicity, and feasibility. These technologies are also described below with brief summary statements under each technology heading.
Candidate methods for the determination of proteinuria

Urine consists of two primary groups of proteins. Albumin is the majority of plasma proteins that cross the filtration barrier and is the predominant urinary protein in most proteinuric kidney diseases. Uromodulin, also known as Tamm-Horfall protein (THP), is the major component of the nonplasma urinary protein and originates in the renal tubules or urinary tract. Other minor subgroups are immunoglobulins, low-molecular weight proteins, and light chains. In general, methods for the determination of proteinuria measure the levels of total protein or its major constituent total albumin. Specific urine biomarker assays, however, measure the concentration of a specific protein related to PE.

1. Gold standard: total protein determination by 24-hour urine collection

Timed urine collection is the gold standard and is very accurate for detecting and measuring proteinuria. However, these assays require central laboratory services, and collection is highly prone to errors.

As mentioned above, the “gold standard” for measuring proteinuria requires a 24-hour urine collection to directly determine the daily total protein or albumin excretion. More recently 2-, 8- and 12-hour collections have been validated. Such collections (typically during a minimum two-night hospital stay) are resource intensive and require refrigeration of the urine during collection and laboratory-based analysis. The gold standard is measuring both protein and creatinine, which is typically done using the benzethonium chloride assay (BCA) and Bradford protein assay for analysis. These are chemical laboratory tests, and not widely available in LRS. In fact, the number of 24-hour collections considered incomplete during studies of pregnant women approach 50%, more than double those in studies of nonpregnant populations. The inherent variability associated with urinary retention and, therefore, timing errors has even lead to the recommendation that pregnant women be hydrated and positioned in lateral recumbency for 45 minutes or one hour before starting and before completing 24-hour collection. As a result, timed collections, while useful in the context of clinical studies and as a reference during establishment of alternative PE/E screening methods, are not usually practical as primary screening methods in LRS.

2. Visually read dipstick (protein only)

These tests are of mediocre accuracy for the determination of proteinuria and of only marginal usefulness for PE screening, but they are widely available, and inexpensive.

The visually read urinary dipstick for protein is a semiquantitative colorimetric test that primarily detects albumin (see Figure 1). It is widely used and inexpensive, costing less than US$0.10 per strip test. Significant proteinuria, however, cannot be accurately detected or is excluded at a protein concentration of 20–30 mg/dL (the 1+ threshold, see Table 1) with point-of-care urine dipstick analysis. The threshold for proteinuria with urine dipsticks is, therefore, around 100 mg/dL (2+ threshold).
Additional challenges to the urinary protein-only dipsticks include poor correlation with protein analysis in 24-hour urine samples, inaccuracy due to the hour-to-hour variability of protein excretion, inaccuracy if the urine is dilute or concentrated or has an abnormal pH, dependency on observer interpretation, contamination by bacteria or blood or vaginal secretions, the relative broad specificity of tetraphenol blue for all proteins, and the susceptibility of dipsticks to deterioration from humidity and temperature. As a result of these issues, evaluations of protein-only dipsticks have shown them to have a mediocre clinical sensitivity of less than 68% for proteinuria detection. A prospective study found that the false positivity for proteinuria (truly negative samples resulting as test positive) ranged from 7% at the 3+ level to 71% at the 1+ proteinuria level, while false-negative (truly positive samples resulting as test negative) rates were 7% for “nil” and 14% for “trace” proteinuria. Dipstick proteinuria was, however, significantly more likely to be correct (true positive/false negative when compared with 24-hour collection) if diastolic blood pressure was elevated > 90 mmHg (p = 0.032) and in the absence of ketonuria (p = 0.001).

3. Visually read ratiometric dipstick

*These tests have increased accuracy but are limited in availability and are expensive for LRS.*

The concentration of protein in the urine may be compared to the creatinine level in a spot urine sample. Protein:creatinine (Pr:Cr) uses urine creatinine as a surrogate marker to correct for variations in daily urine volume. This is used in some institutions instead of a timed urine collection. Studies have
demonstrated that measurement of Pr:Cr in a spot urine sample accurately reflects 24-hour urine collection for detection and quantification of protein in the urine and is simpler, faster, and a more useful method for the diagnosis of significant proteinuria.\textsuperscript{18,19} In addition, there is potential for use as a point-of-care test in facilities that do not have reliable electricity or a laboratory.

**Multistix® PRO strips**

The Multistix® PRO strips (Siemens), specifically the Multistix® PRO 10LS is one of two urinalysis products on the market providing Pr:Cr.\textsuperscript{‡} Among the ten pads (“features”), three pads are used for protein and creatinine ratios: (1) the protein-low pad, specific for protein concentrations from trace to 15 mg/dL, (2) the protein-high pad ranging from 30 mg/dL to greater than 2,000 mg/dL, and (3) a pad for establishing urinary creatinine levels (from 10 mg/dL to 300 mg/dL). In an evaluation of 881 freshly voided urine samples, the Multistix® PRO 10LS demonstrated a sensitivity and specificity to proteinuria (when ratioing the Pr:Cr values) around 75% and 96.5%, respectively.\textsuperscript{20} The study, however, did not examine the correlation to PE. Currently at a cost of US$7.50 a strip, the Multistix® PRO is not economically feasible for LRS.

**Defeatured Pr:Cr dipstick (LifeAssay Diagnostics, South Africa)—in development**

A “simplified” ratiometric dipstick for greater ease of evaluation is projected to have equal accuracy to commercially available Pr:Cr dipsticks, while being comparatively inexpensive. Currently available ratiometric dipsticks are economically unfeasible for use in LRS. PATH has identified a manufacturer in South Africa—LifeAssay Diagnostics—developing a Pr:Cr strip test for economical use in LRS. LifeAssay Diagnostics is an International Organization for Standardization (ISO)-compliant manufacturer of diagnostics for a variety of diseases in strip test format or lateral flow assays in cassette format (see Figure 2). Unlike the Multistix® PRO, which includes seven other analyte pads, LifeAssay can produce a simplified dipstick having two or three pads only for protein and creatinine analytes. Early cost estimates suggest that the product will be between US$0.05–US$0.15 per strip. The LifeAssay Pr:Cr dipstick could be included in an upcoming major clinical study, Pre-eclampsia Integrated Estimate of RiSk or miniPIERS (part of a larger trial known as PRE-eclampsia-Eclampsia Monitoring, Prevention and Treatment or PRE-EMPT) in 2014. Verification testing of the simplified dipstick prototype is being planned for early 2014 to verify the dipsticks prior to formal

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{lateral_flow_cassette.png}
\caption{Example image: lateral flow assay in cassette format (in conceptual development by PATH).}
\end{figure}

\textsuperscript{‡} Multistix PRO and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. Additional information is available at: http://usa.healthcare.siemens.com/point-of-care/urinalysis/urine-test-strips-multistix-pro.
use in the clinical study. If verification is successful, the LifeAssay dipstick could be part of the clinical trial’s routine screening methods of proteinuria. The clinical trial, with a recruitment of nearly 2,100 women, is planned for Brazil, Fiji, Pakistan, South Africa, and Uganda. The data will be correlated to maternal and neonatal outcomes. Successful validation, as measured by improved performance and ease of use, will be convincing evidence for implementation as a replacement to the common protein-only dipstick in current pregnancy care algorithms. Furthermore, as a low-cost, highly portable solution, the LifeAssay ratiometric dipstick could potentially impact task-shifting to community health care workers and enable reaching more isolated pregnant women in rural areas.

Reagent-limited dipstick—in conceptual development by PATH

This conceptual dipstick assay would improve accuracy and reproducibility through a binary call of test results.

PATH has conceptualized a dipstick assay where the need for evaluation of subtle color changes is eliminated. Such a test would contain reagent-limited detection pads wherein graded levels of protein and creatinine are laid out together on a single dipstick. Each pad, whether for protein or creatinine, would measure specific threshold concentrations and thereby result in a binary change of indication color. Instead of a subjective evaluation of color shading, the user would observe a single color change that would correspond to a set threshold concentration. Observation of successive color levels indicating increasing protein and creatinine concentration would then result in the proteinuria status. This concept may help reduce the inherent variation arising from interpretation of colorimetric changes and thus stabilize the range of accuracy values associated with ratiometric dipsticks.

4. Machine dipstick readers (ratiometric)

These test results have only a modest increase in accuracy but an advantage in data connectivity.

Clinitek® and Aution® PocketChem dipstick readers

Sophisticated methods to read dipsticks, such as an automated dipstick reader (Clinitek® [Siemens], Germany; and Aution® [ARKRAY], Japan§—see Figure 3), may increase precision of evaluating colorimetric changes but require reliable electricity, trained staff, and expensive equipment and are, therefore, not suitable for most LRS. In general dipstick readers demonstrate a modest improvement in accuracy.1,21 While most studies are based on dipstick evaluations by trained clinical staff in ideal conditions of a laboratory, a reader study might be

§ Aution and all associated marks are trademarks of ARKRAY, Inc. Additional information is available at: http://www.arkray.co.jp/english/products/urinalysis.html.
of greater benefit in variable point-of-care conditions with field care workers with variable training levels, resulting in a greater consistency and accuracy of results. Another advantage of dipstick readers is immediate connectivity to health management systems through the Internet. For rural locations, even peri-urban locations, the connectivity would provide a link to care with physicians or for ready referral of patient data in care management.

**uChek™ urine Analyzer**

The uChek™ urine analyzer, made by Biosense Technologies**, is a promising reader adapted for use in LRS. The highly portable kit is complete with an illuminator box (Cuboid), a dipstick holding tray (Color Mat), mobile phone, and dipsticks. The Cuboid and the Color Mat provides a stable platform for photometric data collection by supporting consistent lighting and motion stabilization, hence a greater precision of results determination. Data gathering, analysis, and storage programs are already uploaded in the cell phone software. Furthermore, results can be transmitted by wireless networks to clinical services or health management systems unlike other readers requiring hard line/computing infrastructure. While the uChek™ system is less expensive than comparable readers, the cost of the system, US$300, remains high for LRS.

5. **Point-of-care non-dipstick readers (ratiometric)**

These non-dipstick readers deliver high accuracy, but with higher user training requirements and at higher cost.

At the most sophisticated end of the point-of-care technology spectrum are all-in-one analyzers (DCA Vantage® [Siemens], US; and Afinion™ systems [Alere, Inc./Axis-ShieldPoC AS], UK) capable of performing chemical assays utilizing antibody-specific binding of proteins. A small sample of urine, typically less than 5 microliters, is placed in a disposable cassette (Figure 4) that contains anhydrous-reactive chemicals.

Upon rehydration with the urine sample and insertion into the analyzer, the analyzer evaluates the resulting color change or

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**uChek** is a trademark of Biosense Technologies Private Limited. Additional information is available at: [https://sites.google.com/site/ucheksystem/how-it-works](https://sites.google.com/site/ucheksystem/how-it-works).
immuno-turbidity change. Despite the capability of high performance for detecting proteinuria and increased high throughput, the cost of even the smallest-capacity analyzers, US$2,500, far exceeds feasibility at the community level, making them more appropriate for high throughput at established medical institutions.

**Specific urine-based candidate biomarkers for preeclampsia**

Due to inherent lack of sensitivity and specificity of screening for PE by testing for proteinuria, a wide variety of biomarkers have been proposed for diagnosing women with PE and identifying women at high risk of developing PE. Research to uncover biomarkers for PE/E is rich and chiefly targeted to discovery of signature markers in blood†† (to be discussed in greater detail in a separate landscape). If proven effective, dipsticks and other ready-to-use methods to detect blood-based biomarkers could be developed for use at point of care.

Urinary biomarkers, however, present an advantage in that urine samples are more easily obtainable, pain free, in sufficient volume, and are neither infectious nor a biohazard in disposal. The payoff would be high if a urine biomarker or a set of biomarkers could diagnose and predict PE/E or screen for the onset of PE/E. Some biomarkers in urine may be present at high concentrations. They could likely be detected with simple diagnostic rapid strip tests. This translation into lateral flow cassette format (see Figure 3 above) is not only a proven diagnostic platform but also a reliable, economically feasible format for low-resource countries. While some of these biomarkers appear promising, they remain investigational. Several urine biomarkers are described below.

1. **Biomarker of misfolded protein: Congo Red dye assay**

   *Early study results using this assay show promising results for prediction for PE.*

   Protein research work conducted by Irina Buhimschi’s laboratory (while at Yale University) on urine from women with severe PE identified the unique occurrence of aggregated fragments as a possible consequence of misfolded proteins.22 Congo Red is an azo dye which can non-reversibly intercalate within misfolded protein complexes.23 The Buhimschi team is evaluating the use of the dye as dot blots on non-specialized, plain paper24 as a means of a low-cost, usable method in LRS. In a 2009 abstract,25 Dr. Buhimschi’s group applied the Congo Red Dot (CRD) test, as well as blood-based biomarkers, serum indicators, sFlt1/P1GF, and protein to creatinine ratios, to 347 pregnant women across five groups: 1) a normotensive control group, 2) a chronic hypertension group, 3) a gestational hypertension group, 4) a mild PE group, and 5) a group of women with severe PE. CRD test results were correlated to an outcome

†† The UNDP/UNFPA/WHO/World Bank Special Program of Research, Development and Research Training in Human Reproduction (HRP), in collaboration with the Perinatal Research Branch of the National Institute of Child Health and Development, is currently conducting a multicenter study to test whether changes in serum and urinary angiogenic proteins can be used as an effective method for identifying women at high risk of developing PE.
of preterm delivery as a consequence of PE. Plots attached to the short abstract appear to show 1) CRD can differentiate PE from non-PE hypertensive conditions, 2) an indication between a positive CRD test and preterm delivery, and 3) marked higher performance in accuracy over sFlt1/P1GF and protein to creatinine ratios. The Buhimschi group has received a 2013 Saving Lives at Birth award (USAID) to expand work on the CRD.

2. **Tamm-Horsfall protein urine biomarker**

*While in conceptual stage, this biomarker shows promise for prediction of PE, potentially higher performance metrics, and adaptability to a lateral flow assay.*

Abnormal proteinuria is classified into three categories of generalized origins: glomerular, tubular, and overflow. About 40% of urinary proteins are glomerular in origin and the rest are tubular, mainly composed of THP. THP, also known as uromodulin, is a naturally occurring glycoprotein produced by the long tubes of the Henle’s loop. THP is naturally excreted. While the exact purpose of the protein is unknown, two roles are hypothesized: 1) as an inhibitor of crystallization of calcium and 2) as a potent immune suppressant in pregnant women. In 1989, researchers characterized a differential rate of excretion between pregnant women, nonpregnant women, and pregnant women with PE. According to the paper, a significant drop in THP excretion was found in pregnant women with PE. While normal pregnancy is typified by an increase in THP excretion, greater than 51 mg/g creatinine, nonpregnant women had a median THP excretion rate of 20 mg/g creatinine and hypertensive pregnant women with PE exhibited a low THP excretion rate of 9 mg/g creatinine.

Using proteomics in a study of urine protein, THP was one of a few protein groups characterized in the increase of renal disease and hypertension. David Carty (University of Glasgow) used conventional capillary electrophoresis and mass spectrometry to longitudinally examine urine samples from pregnant women from gestational weeks 12 to 16, 20, and 28. From comparison to nonpregnant controls, the authors were able to develop a model of 50 urine biomarkers which increased markedly across the sampling time points for urine samples taken from women who later developed PE. The 50 biomarkers were identified as protein fragments of fibrinogen alpha chain, collagen alpha chain, and THP. In another study, the investigators have identified one fragment of THP, near the promoter region of UMOD gene, which is associated with hypertension independent of renal function. As a result of that work, the authors speculate that THP, “as a key player in renal disease and hypertension, could point toward subclinical vascular and renal damage in the early stage of preeclampsia that are indicated by differential urinary expression of THP fragments and warrant further investigation.”

Anti-THP antibodies are already commercially available, and yet there is a surprisingly low number of studies further investigating THP in pregnant women. A focused investigation in how THP levels vary with pregnancy would inform the possible development of a lateral flow assay.

3. **SERPINA1 urine protein biomarker**
Also in the conceptual stage, this biomarker shows promise for prediction of PE, potentially higher performance metrics, and adaptability to a lateral flow assay.

Dr. Buhimschi and her colleagues also published a proteomic analysis of urine from pregnant women using mass spectrometry of a specialized technology, surface-enhanced laser desorption/ionization (SELDI) technology. Interestingly their analysis uncovered a characteristic fingerprint of SERPINA1 protein (SERine Protease-inhibitor A1) and albumin protein fragments. A comparison of proteomic profiling versus blood-based sFLt-1 to PIGF ratio or urine protein to creatinine ratio showed a much higher accuracy for predicting PE and correlated to a surrogate outcome of mandated delivery. Since SERPINA1 has been tied to pathophysiology of liver damage and encephalopathy, the authors hypothesize that the supramolecular aggregates of misfolded SERPINA1 may be a result of abnormal liver and neurologic changes which arise with PE.

4. C5b-9 urine protein biomarker

Potentially the most clinically sensitive and specific urine biomarker for PE to date.

C5b-9 plays an important role in inflammatory and cell-killing processes. Recently, in December 2013, Burwick, et al. (Brigham and Women’s Hospital, Harvard University) associated the excretion of C5b-9 complement factor to 96% of cases with severe PE, 12% of controls with chronic hypertension, and 8% of healthy controls. Complement activation is part of the systemic inflammatory response to the fetus. In order to protect itself from the maternal immune response, the fetus produces complement regulatory proteins, inhibiting the complement activation cascade. The group hypothesizes that the excess presence of complement factors in plasma during severe PE abets kidney injury, leading to increased concentration in urine excretion. While all the complement factors were found to be excreted in increased amounts in severe PE, urinary C5b-9 stands out dramatically in distinguishing severe PE from chronic hypertension. As with other urine biomarkers, C5b-9 warrants further investigation in proof-of-concept studies, validation, and feasibility. These development activities would be geared to translation for use in LRS as a lateral flow assay.

Investment opportunity

PATH recommends the following opportunities for near-, intermediate-, and long-term investments to create improved tools for proteinuria screening and/or PE diagnosis:

1. Near term (1-3 years): Validate and commercialize LifeAssay’s defeatured Pr:Cr dipstick

Despite the shortcomings of ratiometric dipstick tests, investment in the validation of the LifeAssay dipstick could enable commercialization and scale-up of a near-term, affordable test for LRS.

‡‡ Soluble fms-like tyrosine kinase 1 (sFLt-1) and placental growth factor (PIGF) are both angiogenic proteins identified as blood-based biomarkers for PE.
Specific activities could include:

- Funding the inclusion of the LifeAssay test in the highly strategic mini-PIERS multi-country clinical study planned for 2014. A funding gap to cover the costs for study supplies and labor, especially for the use of gold-standard comparator tests alongside the LifeAssay test remains. LifeAssay has already agreed to provide its tests at virtually no cost for this study.

- Commissioning a research study to include user assessment, monitoring, and evaluation (in parallel to Piers). This study could be designed and prepared right after the Piers data are available.

- Supporting global and in-country advocacy with public-sector stakeholders for adding this test to existing guidelines, government tenders, etc. (once the data are available).

- Supporting marketing activities to public-sector customers by providing in-kind marketing expertise.

The LifeAssay defeatured Pr:Cr dipstick could be used with Biosense’s uChek™ reader to further increase accuracy of interpreting the test, and results could be linked to mobile-based data management systems. However, the many system changes required of a bundled product offering and the overall implications for additional equipment, distribution, and training should not be underestimated. These additional implications and trade-offs were clear when putting the LifeAssay defeatured Pr:Cr dipstick combined with the uChek™ reader through the Strategic Prioritization tool (where it scored lower than the LifeAssay dipstick alone).

2. **Intermediate term (3-5 years): Build the next-generation Pr:Cr dipstick to improve performance and usability**

As a next-generation dipstick, PATH recommends advancing the defeatured Pr:Cr dipstick concept further by developing a reagent-limited, dual dipstick for both protein and creatinine with threshold-limited indicator fields. This will make it easier to accurately interpret results. A one- to three-year development window from proof of concept to clinically evaluated prototype is anticipated.

Specific activities could include:

- Conducting proof-of-principle research and development for reagent-limited, dual dipstick.
- Evaluating reagent-limited, dual dipstick with clinical samples.
- Commissioning a research study to include user assessment, monitoring, and evaluation.
- Supporting global and in-country advocacy with public-sector stakeholders for adding this test to existing guidelines, government tenders, etc.

3. **Long term (4-7 years): Develop rapid assays for PE-specific urine biomarkers on a lateral flow assay platform.**

While further clinical studies are needed, there is the potential that PE-specific urine assays could be developed by creating lateral flow-based assays for one or several of the biomarkers listed above. A biobank, managed by Jim Roberts at the University of Pittsburgh, of over 2,000 well-characterized urine
samples from pregnant women could be available for validation of a biomarker assay. Translation into a lateral flow assay, followed by verification and field validation, would be a longer-term project and could be performed by PATH.

Specific activities could include:

- Conducting proof-of-principle research and development, laboratory validation of rapid strip test, and field validation of rapid strip test.
- In parallel, conducting longitudinal clinical studies with biomarkers to confirm timing of their clinical predictive validity.
- Creating a commercialization strategy, including finding a commercialization partner.
- Facilitating market development.
- Supporting global and in-country advocacy with public-sector stakeholders for adding this test to existing guidelines, government tenders, etc.
References


