Candidate blood-based biomarkers for preeclampsia testing

Technology Opportunity Assessment

Prepared for the
Merck for Mothers Program
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# Acronyms and abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANC</td>
<td>antenatal care</td>
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<tr>
<td>ASSURED</td>
<td>Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable to end-users</td>
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<tr>
<td>BioPE/E</td>
<td>a systematic, large-scale longitudinal study to assess the most promising biomarkers</td>
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<td>BP</td>
<td>BP</td>
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<td>CA-125</td>
<td>cancer antigen 125</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>FDA</td>
<td>US Food and Drug Administration</td>
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<td>GA</td>
<td>gestational age</td>
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<td>GD</td>
<td>gestational diabetes</td>
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<td>glyFN</td>
<td>glycosylated fibronectin</td>
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<tr>
<td>HAPO</td>
<td>Hyperglycemia and Adverse Pregnancy Outcome</td>
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<td>HPV</td>
<td>human papillomavirus</td>
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<tr>
<td>ICS</td>
<td>immunochromatographic strip</td>
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<tr>
<td>LRS</td>
<td>low-resource settings</td>
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<tr>
<td>NGAL</td>
<td>neutrophil gelatinase-associated lipocalin</td>
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<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>PE</td>
<td>preeclampsia</td>
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<tr>
<td>PE/E</td>
<td>preeclampsia and eclampsia</td>
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<tr>
<td>PI GF</td>
<td>placental growth factor</td>
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<tr>
<td>POC</td>
<td>point-of-care</td>
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<tr>
<td>Pr:Cr</td>
<td>protein-creatinine ratio</td>
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<tr>
<td>RDT</td>
<td>rapid diagnostic test</td>
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<tr>
<td>sEng</td>
<td>soluble endoglin</td>
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<tr>
<td>sFtL-1</td>
<td>soluble fms-like tyrosine kinase 1</td>
</tr>
<tr>
<td>START</td>
<td>Screening Technologies to Advance Rapid Testing for Cervical Cancer Prevention</td>
</tr>
<tr>
<td>START-UP</td>
<td>Screening Technologies to Advance Rapid Testing for Cervical Cancer Prevention—Utility and Program Planning</td>
</tr>
<tr>
<td>UA</td>
<td>uric acid</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Statement of need

Preeclampsia (PE) is a life-threatening disorder that occurs only during pregnancy, childbirth, and the postpartum period. It is characterized by high blood pressure (hypertension) and protein in the urine (proteinuria). Convulsions (fits) with signs of PE indicate eclampsia, although convulsions occasionally 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 account 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. 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.

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) 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 levels. ANC utilization is around 68% in low- and middle-income countries compared to 98% in high-resource settings. In fact, more than half of all births occur at home, and many pregnant women receive no prenatal care. Furthermore, the need for urgent 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 30% to 50% or more of these women die.

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 laboratory-based analysis. In fact, the number of 24-hour collections considered incomplete during studies of pregnant women approaches 50%, more than double the rate in studies of nonpregnant populations.

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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.
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 outside of 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 criteria7 (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 reduce morbidity and mortality in PE/E, the urinalysis device should not only improve the accuracy of proteinuria measurements but 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 will 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 covers blood biomarkers that are candidates for PE screening. A previous PATH report, *Proteinuria Measurement and Urine-Based Markers for Preeclampsia Diagnosis*, summarizes urine-based biomarkers for PE screening.

Experts have long sought biomarkers for PE that are sensitive, specific, and reliable; that can be measured with precision; and that allow an objective assessment of the disease. Furthermore, because the presence of increased urinary protein is usually a late manifestation of PE,8 experts have hoped that use of biomarkers would enable earlier detection or even prediction of the disease. An ideal biomarker for PE would allow an accurate prediction during the first trimester, providing a wide window of opportunity for effective treatment that may lead to complete recovery or reduction of disease severity.9

The cardinal clinical features of PE are hypertension and proteinuria occurring after 20 weeks of gestation in previously normotensive women. Although the detection of proteinuria is one of the formal diagnostic criteria for PE and 24-hour urine collection is the current gold standard method for measuring proteinuria, this method is by itself a less-than-perfect predictor.

The ideal diagnostic test for PE in low-resource settings (LRS) would be a low-cost, instrument-free, disposable assay measuring a single, highly accurate and predictive blood biomarker that requires no additional clinical information about the patient (e.g., no blood pressure and proteinuria measurement, no maternal medical history, etc.). Because PE is a complex and probably multifactorial disorder, it is unlikely that a single marker will prove to be an accurate predictive tool.10 Therefore, a more realistic approach would be to combine one or several blood biomarkers with biophysical parameters and clinical information on patient history and risk factors to determine the risk of developing PE.
Two automated immunoassays specific for soluble Fms-like tyrosine kinase-1 (sFtl-1) and placental growth factor (PlGF), (Elecsys® sFlt-1/PlGF from Roche and the Alere Triage® PlGF test) have become available for clinical use to diagnosis PE. Although these two tests have not yet been approved by the US Food and Drug Administration (FDA) for commercial use in the United States, a direct comparison between Triage® PlGF and Elecsys® sFlt-1/PlGF found a clinical sensitivity and specificity of 100% and 96% for Triage® and 64% and 100% for Elecsys® in diagnosing early-onset PE. Although these tests offer hope for improved PE screening, they are not in their current form suitable and affordable for use in LRS. However, both sFtl-1 and PlGF could be used as the basis for simpler rapid assays, probably with less sensitivity and accuracy.

**Scenarios of use**

The test for a biomarker for PE would need to be integrated into the current model of interventions during ANC and with minimal disruption to increase the likelihood that the test will actually be ordered and that the results will influence the individual woman’s care plan. Two likely scenarios for use of a PE biomarker are summarized below:

- **Used as a predictive biomarker in the first trimester of pregnancy.** Ideally, a predictive screening test for PE could be performed early in ANC, preferably at 8 to 12 weeks of gestation. One possibility is to screen all pregnant women at their first ANC visit or develop an algorithm to decide which women would most benefit from the test, implying that the test is available at all points of care where ANC is offered. Because the biomarker in this scenario is being used to predict PE, proteinuria would continue to be an important diagnostic criterion for PE.

- **Used to confirm/rule out diagnosis of PE.** In this scenario, the biomarker could theoretically replace proteinuria in the definition of PE. The potential advantages of this approach include improvement in the sensitivity and specificity of the diagnostic criteria for PE/E, the ability to differentiate between PE and other disease entities that present with proteinuria and/or hypertension, and the ability to predict adverse outcomes. Use of a biomarker in this way would require clinical decisions at two levels: (1) a decision to order the test and (2) interpretation of the results. The latter could be influenced by the gestational age (GA), especially if the biomarker level varies by GA, and by confounders present in pregnant women that may affect the biomarker level.

**Appropriate test formats for preeclampsia screening for low-resource settings**

Non-instrumented, single-use, point-of-care (POC) tests are particularly beneficial in health care settings in LRS. Rapid diagnostic tests (RDTs), generally based on immunochromatographic strip (ICS) or lateral flow technology, are one of the very few diagnostic technologies successfully used in the developing world. Because they rely on inexpensive, off-the-shelf components and reagents, they are affordable, in many cases costing less than US$1 to the end-user. Some ICS assays can achieve analytical sensitivities for proteins to the level of pg/mL, though most can detect only to the level of ng/mL.
Dipsticks, another assay format that does not inherently require a reader, are generally less sensitive than an ICS test and are better suited for detecting macromolecules, such as protein and creatinine in urine. Dipsticks tend to be even cheaper than lateral flow strips, sometimes costing only 5 cents or less per assay.

Microfluidic immunoassay platforms (multiplexed tests) also hold promise for developing simple-to-use diagnostic tests that would enable the testing of more than one PE biomarker at a time.

In addition, low-cost, instrument-free or minimally instrumented microfluidic enzyme-linked immunosorbent assay (ELISA) formats are also becoming available. These formats may prove especially useful for multiplexed PE biomarker assays that require somewhat higher analytical sensitivities in the low pg/mL range.\textsuperscript{12}

At present, the PATH landscape team believes that ICS tests are the most appropriate format for most biomarkers, especially those presenting at clinical concentrations in the low to high ng/mL range in blood or serum. Some urine biomarkers occur at concentrations higher than that and could be measured with dipsticks. For biomarkers presenting in the pg/mL range and below, novel assay formats such as microfluidics-based ELISAs would have to be employed because dipsticks and ICS tests do not have the required sensitivity to detect them semiquantitatively.

All of these formats can benefit from the use of cell phone cameras as readers to improve reading accuracy, minimize user error, and allow linking to a health information system and patient records. While dipsticks and ICS tests can generally be read visually as well as with a reader, ELISAs are generally read with an instrument. Several groups, including PATH, are currently involved in diagnostics research and development to advance the use of standard cell phone cameras combined with custom cell phone software applications, without additional supporting equipment, as reader instruments for diagnostic disposables.

**Review process**

To identify PE/E biomarkers, the landscape team identified 129 unique biomarkers and biomarker combinations from published articles (PubMed) and patents (US and EU). All studies were published between 2008 and 2013. Review articles were excluded because they do not present original research. Seventy-nine biomarkers were subsequently excluded from further consideration on the basis of various criteria, as shown in Figure 1.
Among the 50 biomarkers identified for further consideration, 28 are single biomarkers and 22 are described for use in combination. Two of these 22 combination biomarkers are also used as single markers (cancer antigen 125 [CA-125] and neutrophil gelatinase-associated lipocalin [NGAL]).

The 50 identified markers are referred to in this document as experimental markers. Four biomarkers known to be associated with PE—namely, sFlt-1, vascular endothelial growth factor (VEGF), PIGF, and soluble endoglin (sEng)—were also included in our analysis and are referred to as established markers. To our knowledge, there is no RDT using any of these four well-characterized biomarkers on the market or in development.
Of note, among the studies describing the 50 experimental biomarkers, some used varying criteria to define PE. The conditions for PE as defined in this landscape were adapted from the WHO criteria as follows:

- **Mild PE:** Two readings of diastolic blood pressure (BP) ≥90 but <110 mm Hg 4 hours apart after 20 weeks of gestation with proteinuria up to 2+ or >0.3 g/24 h urine collection.

- **Severe PE:** Diastolic BP ≥110 mm Hg after 20 weeks of gestation with proteinuria ≥3+ or >5 g/24 h urine collection or any one of the signs/symptoms of severe PE (headache, blurred vision, oliguria, epigastric pain, right upper quadrant pain, and pulmonary edema).

- **Early onset of PE** was defined as occurring before 32 to 34 weeks of pregnancy.

**Candidates for preeclampsia testing**

The 28 single experimental markers and 4 established markers noted above in Figure 1 are involved, for example, in placental tissue damage, oxidative stress, renal function, or inflammation. Among the 28 experimental biomarkers, 9 have been shown to have some degree of clinical sensitivity and specificity to PE when compared to blood pressure and/or proteinuria and could be of interest for future PE screening tests. Another 10 experimental biomarkers had limited clinical evaluation information. No clinical data were found for the remaining 9 markers which have been described only in patents for which clinical and performance information is more limited than in the scientific literature.

The 9 promising single experimental blood or serum biomarkers together with the 4 established ones were ranked according to (1) characteristics related to performance and (2) other characteristics, such as whether a kit or commercial antibodies exist (see Table 1). The total number of points a marker could receive was 12. If a marker scored from 1 to 4 points, it was ranked as poor; from 5 to 8 points, adequate; and from 9 to 12, good. The result of this scoring is that 6 of the 13 biomarkers were categorized as good, 2 as adequate, and 5 as poor for predicting PE. There are a few caveats to this classification since the studies describing these markers are not necessarily comparable (e.g., differences in sample size, gestational age, and clinical definition of PE).
Table 1. Overall performance of 13 blood biomarkers in predicting preeclampsia.

<table>
<thead>
<tr>
<th>Ranking category</th>
<th>Biomarker name</th>
<th>Detection level</th>
<th>Sensitivity to PE</th>
<th>Specificity to PE</th>
<th>Clinically relevant</th>
<th>Use in 1st trimester</th>
<th>Researcher/Company/University</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>Glycosylated fibronectin (glyFN)</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
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<td>sEng</td>
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<td>sFlt-1&lt;sup&gt;h&lt;/sup&gt;</td>
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<td></td>
<td>Plasma factor VII</td>
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<td>Serum uric acid (UA)</td>
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<td>Adequate</td>
<td>NGAL</td>
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<td>soluble ST2 (sST2)</td>
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<td>Poor</td>
<td>sFlt1-14</td>
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<td>CA-125</td>
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<td>Glucose regulated protein 78 (GRP78)</td>
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<td>Hydroxysteroid (17-beta) dehydrogenase 1 (HSD17B1)</td>
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<td></td>
<td>VEGF</td>
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</table>

<sup>a</sup> Performance of the biomarker in predicting PE.
<sup>b</sup> ≥5 ng/ml.
<sup>c</sup> ≥90%.
<sup>d</sup> Significant difference of biomarker level between control group and PE patients.
<sup>e</sup> Rapid strip assay prototype has been developed.
<sup>f</sup> Antibodies commercially available.
<sup>g</sup> Assay commercially available.
<sup>h</sup> Elecsys<sup>®</sup> sFt-1Roche.
<sup>i</sup> Alere Triage<sup>®</sup> PlGF. Antibodies developed but not commercially available.
GlyFN, even though its role in PE is less documented, has an advantage over the other “good” candidates since an RDT already exists (J. Räsänen, unpublished data, 2013). sEng, sFlt-1, and serum UA are promising candidates as well. Although an automated assay specific for sFlt-1 exits, its current format is not suitable for LRS due to financial and practical concerns. The existing test kit to measure serum UA level requires a mix of enzymes. This means that the kit would need to be kept at a low temperature, which is rather difficult in LRS environments. sEng may prove useful in differentiating PE from other hypertensive diseases of pregnancy.13 As for PlGF, its current level of detection is in the range of 100 pg/mL, which is far from ideal for an RDT.

Overall, six candidates warrant further investigation and validation. Additional activities would be geared to translation for use in LRS, as by a lateral flow assay.

Investment opportunity

Despite extensive research to date, a reliable screening test with high sensitivity, specificity, and predictive value has yet to be developed. Although the biomarkers listed in our tables have been examined in numerous clinical and laboratory studies, many of them have not reported sensitivity and specificity. Some studies have reported promising positive or negative predictive values but with large confidence intervals due to the low number of patients. Other studies have compared assays in women with established disease or have tested at a fixed time point rather than at presentation. Overall, the existing data are in many cases contradictory and confusing. Despite the lack of definitive data on biomarkers, there are some emerging assay development activities that look promising. With the exception of glyFN, none of the biomarker studies to date has considered issues related to the use of any resulting tests in LRS. Issues include the feasibility of developing an LRS-compatible assay (low cost, easy to use, requiring no instrument or only a simple instrument) for a particular biomarker, ethnicity, and typical timing of first and subsequent ANC visits by pregnant women in LRS.

PATH recommends the investment opportunities below to create improved tools for blood biomarker screening for PE diagnosis. These opportunities are part of an overall portfolio of staged PE/E predictive and diagnostic intervention product development and introduction activities.

This portfolio would ultimately include: (a) an initial protein-creatinine ratio (Pr:Cr) urine dipstick test currently under evaluation; (b) an advanced, easier to read version of a Pr:Cr test; (c) an initial biomarker-based rapid assay for either blood or urine, based on one of the biomarkers currently under advanced investigation; and (d) an advanced highly sensitive and specific biomarker test (most likely blood-based, comprising multiple biomarkers), based on biomarkers that still need investigation. In terms of timing, they could be available (a) as soon as 2015, (b) around 2016, (c) around 2017, and (d) closer to 2019, respectively. As more advanced tests become available, the use of earlier versions will likely decrease, although Pr:Cr tests will continue to be beneficial for use in testing for kidney disease and urinary tract infection. The opportunities for development and introduction of Pr:Cr tests are discussed in more detail in the previous PATH landscape on urine-based biomarkers.
At this point, it is not yet possible to assign the proposed products to use categories such as predictive, diagnostic, and prognostic. It is likely that the levels (concentrations in blood or urine) of most biomarkers under consideration will either significantly increase or decrease as pregnancy progresses. An assay that will be able to determine this increase or decrease most accurately (such as expensive and complex ELISAs and proteomics-based assay panels under development for high-resource settings) would potentially become a predictive assay. A less accurate and sensitive test might only be able to determine biomarker level changes once they become significant enough—later in pregnancy—and thus become a useful diagnostic. It is also likely that novel, sensitive, but still relatively inexpensive emerging platforms such as microfluidics-based ELISAs could achieve similar sensitivity and accuracy as the more complex assays but at a lower cost. Additionally, it is possible that some biomarker tests, possibly including the Congo Red urine test, might turn out to be predictive for PE in women pre-pregnancy (although definitive studies on this have yet to be published), but the large majority of biomarkers will only be detectable during pregnancy.

**Short-term opportunities**

1. **Invest in ongoing promising biomarker assay development activities that may yield LRS-compatible tests.**

To create an initial rapid assay based on one of the biomarkers currently under advanced investigation (c. above), we propose investing in two to three biomarker-based assay development approaches that show potential compatibility with LRS.

Despite the lack of definitive data on biomarkers, there are some emerging assay development activities that look promising. The following groups are currently pursuing such tests, though not specifically for LRS. However, the following biomarkers investigated for use or under development in assays by these groups could possibly be used in LRS-compatible assays.

- **DiabetOomics.** This company is developing a POC test for analysis of glyFN levels. This test is currently in an ELISA format, but the company has made some efforts towards developing an RDT version in blood that may facilitate rapid screening in underserved populations.

- **Miraculins Inc.** This company is seeking commercialization opportunities for the biomarker Endoglin. Alere has declined to proceed further with its Endoglin license from Miraculins, and the parties have agreed to work to enhance Miraculins’ rights to receive a secure supply of high-quality, proprietary Endoglin reagents manufactured by Alere.

- **Carmenta Bioscience, Inc.** This company is working on a combination of six markers associated with placental tissue damage, oxidative stress, renal function, and inflammation. Carmenta, in collaboration with researchers at Stanford University, is waiting for patents as of December 2013.

- **IMPROvED consortium.** The IMPROvED (Improved Pregnancy Outcomes via Early Detection) consortium is a new and distinctive partnership of four small and medium enterprises (Pronota NV, MediSciNet AB, Metabolomic Diagnostics Ldt, Accelo5ment AG) and eight academic
institutions. At the end of 2012, IMPROvED launched a Phase IIa prognostic multicenter hospital-based clinical study\textsuperscript{14} using biomarkers previously identified through metabolomic and proteomic platforms for PE prediction.\textsuperscript{15,16}

- **Thermo Fisher Scientist and PerkinElmer.** Both predictive tests from these companies combine fluorescent immunoassays of PIGF, pregnancy-associated plasma protein A, and α-fetoprotein with physical measurements of mean arterial pressure and of the uterine artery Doppler pulsatility index. The test sold by Thermo Fisher Scientific is a kit to be run on its Kryptor\textsuperscript{™} clinical analyzers.\textsuperscript{2} The PerkinElmer test is available in the United States as a clinical testing service performed by PerkinElmer Labs/NTD. Both tests are meant to be used at the end of the first trimester, between weeks 10 and 14. To our knowledge, neither kit has been approved by the FDA for use in the United States.

PATH has existing relationships with Miraculins and DiabetOmics; both small businesses have expressed interest in collaborating on making a LRS version of their assay available. Despite the fact that the biomarker characterizations are not definitive, we believe that both tests would be an improvement over current PE/E screening practices and are worth supporting. Currently, both tests will initially be targeting developed countries, but with some assistance and funding, it is likely that the companies could be persuaded to develop rapid strip test versions of these assays that could then be evaluated in clinical studies.

Proposed activities could include the creation of a target product profile of a LRS-compatible assay based on the prototypes under development by the partners; evaluation of existing prototype assays in PATH’s laboratory with contrived and clinical samples; design input into assay format and chemistry by PATH scientists and engineers, with input from relevant Merck experts; reevaluation of an LRS-compatible prototype; and planning and execution of an initial field study (preferably nested into one of the existing PE/E studies such as miniPIERS (Pre-eclampsia Integrated Estimate of RiSk). The collaborations would be very similar to many other product development projects that PATH has successfully completed in the screening and diagnostics space, including strip tests for cervical cancer screening (with Arbor Vita Corporation), Chagas (Laboratorio Lemos), and Onchoceriasis (Standard Diagnostics, Inc.), as well as more-complex tests for human papillomavirus (HPV) (Qiagen), tuberculosis (Alere), and infant HIV (TwistDx Limited) screening and diagnosis.

### Longer-term opportunities

2. **Create a plan to develop a systematic PE/E biomarker study that investigates factors of relevance for biomarkers for populations in LRS.**

A systematic, large-scale longitudinal study that assesses the most promising biomarkers (known hereafter as BioPE/E) with laboratory-referenced methods and compares them with clinical outcomes, with consideration of patient risk factors, would be very valuable. Ultimately, the question that needs to

\textsuperscript{2} KRYPTOR is a registered trademark of CIS bio international, licensed for use by B·R·A·H·M·S, a part of Thermo Fisher Scientific.
be answered is, “What is the most appropriate combination of biomarker(s), assay format, and use algorithm that balances clinical predictive and diagnostic performance with cost, ease of use, and ease of access in LRS?”

Given the physiological complexity of PE/E, a large, collaborative multisite study (approximately five sites) along the lines of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study would be ideally suited to answer this question. The HAPO study provided an opportunity to revise diagnostic criteria for gestational diabetes (GD); the data generated by the study were used to develop outcome-based criteria for classifying glucose metabolism in pregnancy and also served as the basis for the new threshold values for diagnosis of GD. To date, the HAPO data are significantly influencing the decisions of numerous international health authorities whether to establish a general screening for GD as an obligatory part of prenatal care.

Such a study is likely beyond the capacity of any single research group and possibly even any single funder. It is, therefore, critical to assemble a consortium of researchers, thought leaders, funders, and private-sector parties with interests in PE/E biomarkers and the development of screening and diagnostic tests. This study would ultimately result in consensus regarding a screening and diagnostic algorithm.

PATH has a history in acting as a convener for similar large-scale efforts and is well positioned to create a consortium and a plan for such as study. For example, the Screening Technologies to Advance Rapid Testing for Cervical Cancer Prevention (START), and its follow-up program Screening Technologies to Advance Rapid Testing for Cervical Cancer Prevention—Utility and Program Planning (START-UP) at PATH developed, piloted, and introduced a suite of screening approaches for cervical cancer suitable for all levels of health care in LRS. The program convened several competing companies, WHO, several research groups and ministries of health, and funders (the Bill & Melinda Gates Foundation, National Institutes of Health [NIH], and corporate donors) and resulted in commercialization of an HPV screening test (CareHPV), a cervical cancer rapid strip test (OncoE6), and a now widely used screening algorithm (VIA [visual inspection with acetic acid]). PATH’s role in START and START-UP included leading clinical field evaluations, technical design consulting with assay developers, developing the target product profiles, advocacy, and pilot introduction in select countries.

PATH is ideally suited to act in similar roles as part of the BioPE/E study. We propose an 18-month planning program during which we will:

- Convene a cooperative research group similar to the one formed for the HAPO study. This will not only ensure buy-in but also acceptance of the results in the wider community of experts.
- Engage PE/E biomarker researchers.
- Convene potential funders, including the Gates Foundation, NIH, and the US Agency for International Development.
- Engage interested companies.
- Publish a consensus paper outlining the BioPE/E study, its goals, and a proposed implementation plan.

- Write research funding proposals to interested funders.

While details will be determined during the consultative process during the planning grant, we envisage, similar to the HAPO model, a study that will include approximately 1,000 to 2,000 pregnant women per site that are followed for the duration of their pregnancy and beyond (ideally, a pre-pregnancy blood and urine work-up would also be available). Blood and urine would be taken monthly and tested for approximately 20 blood biomarkers and 3 to 5 urine biomarkers using established laboratory methods. The biomarker levels and trends would then be correlated with patient health history, clinical symptoms as pregnancy progresses, and, ultimately, clinical PE/E and birth outcomes. All sites would use identical clinical definitions and comparable laboratory procedures.

Prior to final selection of candidate biomarkers for the study, we propose holding a workshop with the BioPE/E cooperative research group, including Merck and PATH scientists. With preliminary data (including data from opportunity 1 above), the workshop attendees will also be tasked to determine whether the selected biomarkers would be well suited for predictive screening and/or diagnosis. The levels of biomarkers for predictive screening may be lower than the levels of the biomarker for diagnosis. Different assay formats may be needed for the different uses. For example, a predictive screen test, with lower pg/mL levels for the biomarkers of interest, may need an ELISA format, while a diagnostic test could potentially occur on a rapid strip test. Multiple considerations will be considered for the final selection of candidate biomarkers including the following: preferences for predictive screening versus diagnostic tests, complexity of the likely assay format, suitability for use in LRS, and biomarker performance.

3. **Develop rapid assays for PE-specific blood biomarker(s) selected from the BioPE/E study.**

As the BioPE/E study gets under way, we will expect that promising biomarkers for different LRS use cases (predictive, prognostic, and diagnostic) will emerge. These tools may include biomarkers from the assays we propose to support with the short-term efforts of more advanced biomarkers described above in invest opportunity 1 but may also be biomarkers identified in the larger study as proposed under opportunity 2. It is expected that these tools will primarily be developed by private industry with partial external grant funding, as the BioE/E study will have developed an ecosystem to nurture these new tests by enabling evaluations and pilot introduction as well as advocates for the uptake of appropriate PE/E assays.
References


17. Coustan DR, Lowe LP, Metzger BE, Dyer AR; International Association of Diabetes and Pregnancy Study Groups. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: paving the way for