

## Siemens Foundation/PATH Ingenuity Fellowships

### Project: *New diagnostics may impact health seeking behavior: What is the evidence?*

**Student Requirements:** Graduate student with experience with literature reviews, strong writing skills, ability to synthesize and summarize scientific literature. Knowledge of hypothesis testing, study designs.



**Project Overview and Background:** To drive the uptake and adoption of new health interventions, demonstrating health impact is essential. However, demonstrating the impact of diagnostics is challenging. Diagnostics alone do not impact health outcomes, but rather it is the information they provide that if acted on can impact health. We know that clinician compliance to diagnostic results can decrease the cost-effectiveness of new tools. However, do the presence of new diagnostic tools have favorable effects on health seeking behavior? Are patients more likely to seek care if a simpler, cheaper, less invasive, observable diagnostic is available to provide the health worker and patient with faster and accurate diagnostic results? The first part of this project will include a systematic review of the literature to identify case studies where the availability of new diagnostic tools has impacted health seeking behaviors. Examples may include: prenatal ultrasounds, HIV testing, TB testing, malaria testing. Before launching a new diagnostic test, extensive field evaluations are conducted to determine performance and optimize usability. When thinking about measuring the utility of new diagnostics, what are all the outcomes that a new diagnostic may impact and how can they be evaluated in rigorous study designs. The second part of this project will be to propose a study design to evaluate the impact of new diagnostics.

**Project Objective:** To determine if there is evidence to suggest that the availability of new diagnostic tools has an impact on health seeking behaviors, independent of the performance of the test.

**Student Activities:**

- Report writing up findings from literature review, preferably formatted for publication in a peer-reviewed journal
- Drafted study design to test hypothesis that introduction of a new RDT may impact health seeking behavior for the following diseases: onchocerciasis, lymphatic filariasis, leprosy

### Project: *DIAMETER III: IDT Development Initiative and BIOME*

**Student Requirements:** Undergraduate or graduate student with interest in analytical biochemistry, biophysical chemistry, biology, global health. Previous laboratory training and experience in biochemistry and molecular biology.

**Project Overview:** The aim of the DIAMETER team is to enable access to the most appropriate malaria diagnostic tools to support malaria elimination tactics. Starting in 2012, PATH received funding for the concept and technical feasibility stages for the development of a *Plasmodium falciparum* (*Pf*) infection detection test (IDT). During the concept stage, the DIAMETER team conducted technology landscapes, a user needs analysis, and a market analysis. In close collaboration with the foundation and a technical advisory group, the DIAMETER team identified the histidine-rich protein 2 (HRP2) IDT as a high-priority investment and developed a preliminary target product profile for an HRP2 IDT with 10x improved limit of detection over existing commercial rapid diagnostic tests.

- The IDT Initiative: The IDT Development Initiative aims to enable point-of-care detection of low density *Pf* infections to support malaria elimination. As managing partner for the IDT Development Initiative, PATH is collaborating with partners toward the goal of a successful launch of a highly sensitive HRP2 IDT in 2017.
- Biomarkers for Malaria Elimination (BIOME): BIOME will generate clinical data to better understand the concentration of key *Pf* biomarkers—including HRP2, parasites, and nucleic acids—and their kinetics over the course of an infection. The results will provide a clearer understanding of the potential of a new test for detecting infected individuals with low-density infections.

**Project Objectives:** The student will be involved in determining various relevant physiological, biophysical, and clinical aspects of the *Plasmodium falciparum* HRP2 biomarker for malaria infection detection toward the development of improved point-of-care rapid diagnostic tests for malaria elimination studies.

**Possible Student Activities:** (1) conduct laboratory testing and evaluation of developing alpha and beta prototype IDTs, (2) determine HRP2 concentrations in clinical specimens via ELISA or RDT/IDT analysis and correlate to results from other infection detection diagnostic methods such as microscopy and PCR, (3) usability analysis and compilation of end-user feedback of developing prototypes, (4) engage with other researchers in studies to understand the basic physiological and biophysical characteristics of the HRP2 protein that could lead to increased diagnostic tool sensitivity and specificity.

### Project: *Development of a quality indicator for dried blood spot specimens*

**Student Requirements:** Undergraduate or graduate student. Basic lab skills in ELISA or other immunoassay preferred. Previous handling and/or comfort with handling human biological specimens needed.



**Project Overview and Background:** Dried blood spots (DBS) are widely collected and used and represent a precious specimen source. Unlike plasma and serum, DBS are routinely collected during field surveillance in many countries and as accessory for reference testing when evaluating new

**Diagnostics.** The preservation of the analyte in the DBS is highly dependent on the treatment and storage of the DBS. While everyone agrees that antibody analytes are relatively stable proteins, there is also agreement that it is imperative to store the DBS dry and cold. Many field surveillance activities take place at high temperature and humidity and without cold chain to transport the DBS and unreliable power for longer-term storage. Anecdotal reports of DBS with poor quality have been communicated between researchers. Poor quality specimens could greatly jeopardize a study's accurate results. Many organizations state they have best practices for taking and storing DBS, but not many have transferred procedural information about how to test the DBS quality if handling has not been ideal. Partners at the Centers for Disease Control have suggested using antibody to *E. coli* (to which most people have been exposed), but knowledge of the best target proteins are still needed. Furthermore, more data is needed to know if it can be applied as a diagnostic for reduction in specimen quality. Many researchers and country-wide health programs rely on the results generated from DBS to understand disease prevalence and impact of health programs, but assays may be negatively impacted by poor quality of DBS, leading to loss of valuable data. A sample quality indicator for DBS that is available to PATH and larger community of researchers is greatly needed to help conserve precious health program resources and increase the confidence in the data produced by the hard work from field surveillance teams and laboratory workers.

**Project Objectives:** The student will be involved in identifying candidate quality indicator analytes for DBS with a focus on what is best for antibody testing. The student will then conduct experiments to test the chosen analytes in an assay. The results will contribute to the development of better quality control reagents and improve the standard operating procedures for the handling of DBS, a key clinical specimen for us in diagnostic tests.

**Possible Student Activities:** Literature review will be done to identify candidate universal DBS quality markers. Laboratory testing and evaluation of candidate tests for analytes in assays such as ELISA or RDT will be conducted in order to test for indicator analyte presence. Reality-based scenario experiments that simulate the degradation of DBS using environmental chambers (exposure to humidity and heat) will be designed and conducted.

## Project: *STH diagnostic product development*

**Student Requirements:** Undergraduate or graduate student. Strong analytical and writing skills, ability to work independently and seek assistance when required, flexibility to take on a variety of tasks, and keen interest in technologies and public health. Experience working in a molecular biology/microbiology laboratory. Hands on experience on nucleic amplification assays, extraction and manipulation of DNA/RNA, protein purification, immunoassays.

**Project Overview and Background:** Soil-transmitted helminthiasis (STH) is a neglected tropical disease caused by parasitic intestinal worms. These parasites are spread through contact with soil contaminated with human feces, so STH commonly occurs in places with poor sanitation. As many as two billion people worldwide are at risk of infection, especially in Africa, Asia, and Central and South America. STH causes illness and malnutrition, and children with STH suffer from impaired growth and cognitive development. STH can be controlled with mass drug administration for at-risk populations and prevention activities like improved sanitation and hygiene. Infection levels must be closely monitored to assess the impact of these interventions and guide control programs. However, current diagnostic methods are labor-intensive to perform in the field, and are not sensitive enough to detect low levels of infection, which will become more common as control efforts scale up.



PATH is working with partners to accelerate the development of a rapid, sensitive diagnostic to guide STH control programs. We collaborated with leading organizations in STH research and programming to develop target product profiles describing user needs and technical requirements for new diagnostics. Currently, PATH is communicating these profiles to the diagnostics industry to catalyze the development of STH tests that meet the needs of control programs.

**Project Objectives:** The intern will contribute to novel research, improve his/her skills in basic laboratory work and understand a variety of lab equipment, further his/her knowledge on basic principles of experimental design and testing, and expand his knowledge in several key areas of diagnostics research, specifically in assay development. The intern will work in a busy laboratory, with initial supervision from the performance leader, and eventually fully engage into a key part of the research study.

**Proposed activities:** Assist in the evaluation, development or refinement of rapid diagnostic tests (RDT) or nucleic acid amplification test (NAAT) for soil-transmitted helminths (STH).

1. **Evaluation of immunoreagents for potential use as diagnostic reagents for STH.** Plasmids expressing worm-specific excreted/secreted (E/S) proteins from *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* and *Necator americanus* and the corresponding antibodies against those E/S protein antigens were received from collaborators and will be characterized. Specific activities will include, but not limited to, the production and purification of recombinant protein antigens, purification of polyclonal antibodies, preparation of antibody conjugates and development of ELISA for evaluating the antibodies, and data review and analysis. At the end of the internship program, the intern will provide the results of the evaluation study, and identify which biomarkers will be advanced for RDT development.
2. **Development of multiplex RDT for STH.** Depending on the progress of the RDT development works, the intern will assist in developing a multiplex lateral flow assay for STH using the STH biomarkers selected based on the evaluation study. Activities will include optimization of assay conditions, preparation of lateral flow strips, performance evaluation of the alpha-prototype, and stability testing at different storage temperatures. At the end of the internship program, the intern will contribute to the development of an RDT alpha-prototype for STH.
3. **Lab-based evaluation of NAAT alpha-prototype.** It is hoped that at this point, a small lot of lyophilized STH NAAT reagent has been produced by TwistDx and ready for validation. The intern will assist in lab-based evaluation of the alpha-prototype (NAAT including the refined version of the sample extraction procedure) using actual STH-positive stool samples, compare performance versus "gold standard" method, and gather additional data relating to product stability and usability of the prototype (sample extraction and NAAT).
4. **Development of multiplex NAAT and DNA lateral flow detection.** The intern will assist in the development of a multiplex RPA assay for STH diagnostics by optimizing the concentration of primers and probes, and assay conditions. He/she will also assist in the development of multi-stripe DNA lateral flow strips for detecting DNA amplicons by optimizing the concentrations of capture and detector molecules, etc.
5. **Cost analysis.** Depending on the progress of the STH diagnostic product development, the intern will determine the cost of the test (either NAAT or RDT) as it relates to the ongoing STH control program. Activities will include, but not limited to, itemize resources, data collection on resource costs, data collection on resource allocation, estimation of indirect costs, estimation of total costs, estimation of costs relating to program activities, estimating costs per participant, etc.