Rapid and Quantitative Detection of Malarial Antigen for Microfluidic Point-of-Care Diagnostics in the Developing World

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Abstract

We demonstrate a flow-through membrane immunoassay for malaria on a disposable microfluidic card with integrated dry reagent storage

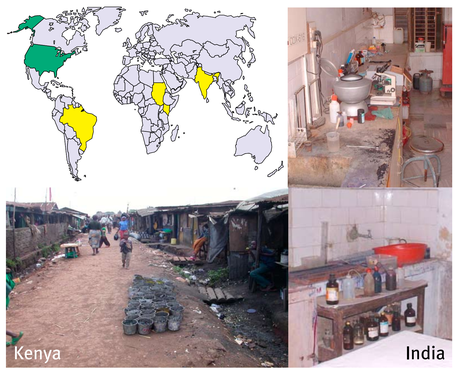
A novel, point-of-care immunoassay format is demonstrated for malarial diagnosis. The assay system consists of a disposable card with integrated dry reagent storage and an external reader that conducts fluidic actuation and assay quantification. Reagents flow through a porous membrane encased in the card's polymeric laminates to conduct a colorimetric sandwich assay on the membrane's surface. The system produces quantitative results with detection limits on the order of ELISA in under 9 minutes.

Global Health Needs

Effective diagnostic strategies for low-resource communities are enabled by stable reagent storage, rapid results, and assay automation

The settings of low-resource communities place additional requirements on diagnostic tools.

- A lack of refrigeration demands stable reagent storage in high and fluctuating temperatures.
- Rapid diagnostic results allow health care workers to diagnose and treat disease in a single visit.
- Inadequate lab facilities and limited health worker training make self-contained and automated assays valuable to obtaining quality diagnoses.



Assay Format Advantages

Reformatting of lateral flow components allows greater assay control, better integration with microfluidic components, and parallel multiplexing

While sharing some materials with lateral flow assays, this design uses pumps and microfluidic channels to direct fluid through the assay membrane in a transverse direction. This format allows:

- Active control of flow rates
- Flexible sequencing of reagent addition
- Simple integration with other microfluidic components
- Parallel multiplexing by spatial separation of different capture molecules on the membrane.

Additionally, the porous structure of the membrane decreases diffusion distances and provides a high surface area for capture of analyte and label. These benefits have the potential to improve assay signal and generate rapid assay results.

Sample-to-Result Disposable

Using a disposable assay card and an external reader, we integrate dry reagent storage, rapid diagnosis results, and lab-on-a-chip automation in an assay for the malarial biomarker *Pf*HRP2

Assay cards were developed with the following features:

- A porous membrane is embedded in laminate construction to conduct a flow-through membrane immunoassay for *Pf*HRP2, a biomarker for malaria.
- On-card storage of solid-phase reagent allows unrefrigerated storage of the disposable.
- A quantitative result in under 9 minutes allows rapid diagnosis of malaria.
- Assay automation provides reproducible results by limiting human error.
- A valveless card design is made possible by simultaneous control of fluid lines.

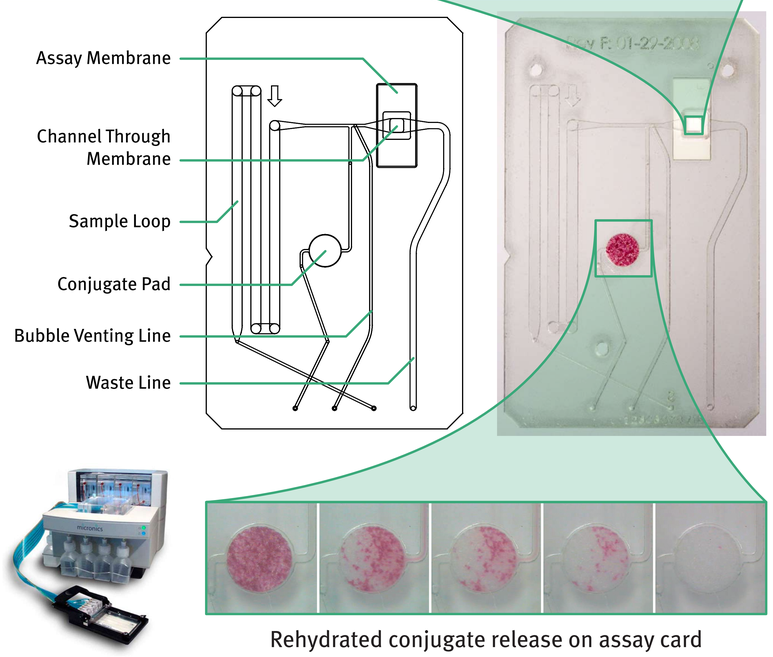


Image: Assay card, pumping system, and video frames from card operation

Flow-Through Membrane Immunoassay Format

Forced flow through a porous membrane gives high surface area and allows control of flow rates

The assay card implements a flow-through membrane immunoassay (FMIA) for *Pf*HRP2 in a sandwich assay format.

- A porous nitrocellulose membrane is patterned with anti-PfHRP2 IgM, and the membrane is held between channels laser-cut in adhesive-backed Mylar laminates.
- Sample is loaded into the card.
- Syringe pumps pass the sample through the membrane, and PfHRP2 is captured on the membrane. Buffer washes away unbound sample.
- Dry anti-PfHRP2 gold-antibody conjugate is rehydrated from a fibrous pad and passed through the membrane. It binds PfHRP2 captured on the membrane, and buffer washes away unbound conjugate.
- Red spots appear with intensities proportional to the PfHRP2 content of the sample at those membrane locations patterned with capture antibodies.
- Assay result images are quantified to determine assay signal.

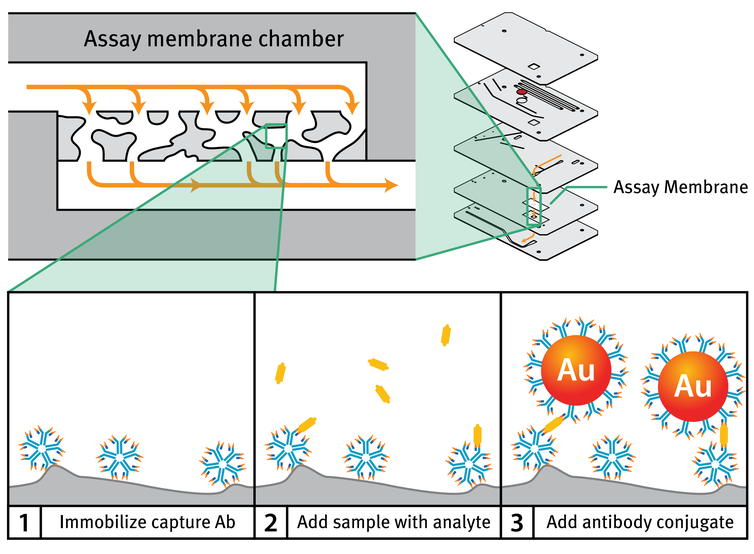


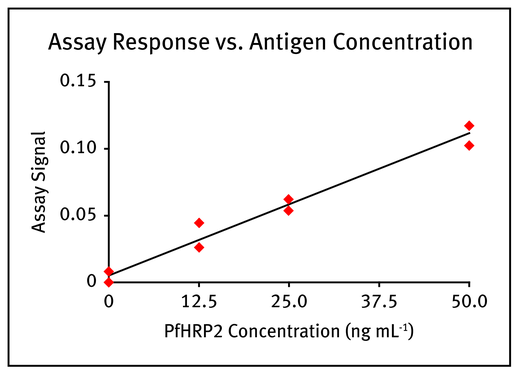
Image: Exploded laminate card, flow-through assay format, and sandwich immunoassay steps

Performance

Assay signal is improved by modifying card geometry and assay timing, giving a detection limit similar to plate-based ELISA (Enzyme-linked immunosorbent assay)

The assay system was improved in multiple design iterations, demonstrating several characteristics.

- The detection limit for PfHRP2 is comparable to a commercial plate-based ELISA and is performed in a fraction of the time.
- Assay signals can be increased by lengthening membrane occupancy time of reagents, such as by reducing flow-through areas and flow rates.
- Taylor dispersion and high density of rehydrated label cause non-uniform exposure of conjugate to the assay membrane. Non-uniform reagent exposure has been addressed by pneumatic pumping approaches and altered protocol timing.



Ongoing Development

Assay multiplexing, pneumatic fluid actuation, and fully-automated assay quantification

This work provides the basis for ongoing improvements to the assay system demonstrated.

- Assay are multiplexed by patterning multiple capture reagents on the membrane. Diseases diagnosed using the FMIA format include malaria, measles, rickettsia, dengue, and typhoid.
- Pneumatic fluid actuation simplifies pumping system requirements and reduces Taylor dispersion.
- Automated image analysis gives repeatable, objective assay quantification.
- Assay cards are being developed for use with a point-of-care diagnostic reader for the developing world, to include immunoassay and nucleic acid assay results from a finger-stick blood sample.

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