Low-cost, multiplexed sensors made from paper

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Healthcare in the Developing World

Challenges
• Patients dispersed in highly-decentralized villages
• Health workers have minimal training
• Portability, disposability is key
• Follow-up is difficult, rare
• Phlebotomy is difficult
• Cold storage often not possible
• Electricity, clean water often not available
• Cost is everything

Similar design constraints apply to environmental monitoring
Introduction to Diagnostics For All
Diagnostics For All (DFA)

- Non-profit, 501(c)(3) organization
- Founded in 2007
- Located in Cambridge, MA
- 15 full-time employees, ~20 part-time volunteers
Exclusive license to paper-based microfluidics from Harvard University (Whitesides Lab).

“Patterned Paper”

- Ubiquitous and low cost
- Common to biological labs/assays
- Fluids readily wicked (without pumps)
- Easily printed on / patterned
- Reagent storage
- “Stackable”
Sustainability to Pursue our Mission

- DFA is pioneering on-purpose innovation specifically for the developing world. We will support our mission through:
  - Project Specific Funding
  - Philanthropic support
  - Licensing opportunities for developed world

Potential Licensing Opportunities

- Pediatrics
- Emergency Response
- Consumers
- Water Testing
- Bioterrorism
## DFA Product Pipeline – Funded Programs

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<td>Human Pregnancy</td>
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Capabilities
Patterned Paper Technology Platform

- Clinical Chemistry Assays
  - Immunoassays
    - Acute Phase Panel
    - STD Panel
  - Metabolic Panel
    - Liver Function
    - Protein
    - Hemoglobin
  - Electrochemical Assays
    - Aflatoxin
    - Sample Concentration
    - Glucose
    - Cholesterol
    - Heavy Metals
- Molecular Diagnostics
  - Fever Panel
  - Multiplexed Combinations
  - HIV
  - Influenza
  - Cancer
  - E. coli
Wax Printing

Wax printing – extremely low cost

- Rapid prototyping
- <1 minute/sheet
- Maintenance and ink* → 0.029 cents/device cost
- Highly scalable

Amenable to large scale manufacturing - Xerox CiPress 500®

Diagnostics For All; All Rights Reserved
4 samples distributed into 64 detection zones.

8 samples distributed into 512 detection zones (compatible with an 8-channel pipette).

PNAS 2008, 105, 19606
Electrochemical Detection

- Quantitative detection on paper devices
- Proven for biological applications (glucose) and environmental applications (heavy metal ions)
- Easy patterning using conductive inks and screen printing

![Graph showing current vs. Pb²⁺ concentration](image)

**Screen-printing of electrodes**

**Integration with low-cost readers**

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Lab Chip, 2010, 10, 3163
Telemedicine Compatible

- The ubiquity of cell-phones in the developing world creates opportunities for rapid response from centralized laboratories.

- DFA’s colorimetric assays can be captured by a camera-phone and analyzed remotely or by the phone itself.

1) Remote processing

2) On-phone recognition & processing

*Anal. Chem. 2008 80, 3699*
Example 1: Liver Function Test
**Problem:** Routine monitoring of liver function is expensive and typically not performed for patients living in resource-limited settings

- Significant liver damage in up to 33% of TB patients receiving therapy
- Significant damage in 13% of patients taking Nivirapine anti-retro viral therapy (HIV)
- Further complicated by other factors (alcoholism, pregnancy, malnutrition etc).
- Co-infection is frequent (e.g. Hepatitis)

**Need:** A low cost, point of care, method of monitoring liver enzymes (AST and ALT) to enhance patient care
Paper-based liver function test

1. Prick finger
2. Apply to device
3. Red blood cells filtered
4. Color-coded results

Processing Time (minutes)
- 0 m
- 0.5 m
- 15 m

AST Read Guide

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<thead>
<tr>
<th>&lt;3X ULN</th>
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ALT Read Guide

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Liver Function Test – Achievements to date

- **ALT** – 95% **accuracy** in bin placement of clinical specimens (>100 samples)
- **AST** – 94% **accuracy** in bin placement of clinical specimens (>100 samples)
- Linear range: 40 – 200 U/L
- Limit of detection: **ALT = 53 U/L, AST = 84 U/L**
- CV’s < 10% for both ALT and AST (low and elevated levels)
- >12 month stability as measured by accelerated studies
- **Interfering Factors**: Pyruvic acid, ascorbic acid at abnormal levels (ALT)
- Manufacturing – able to make **500 devices/day** with current sheet-fed, semi-automated process
- **Field testing underway** – HIV clinic in Ho Chi Minh City, Vietnam – collaboration with Beth Israel Deaconess Medical Center, PATH, Hospital for Tropical Diseases
Liver Function Test - Field Study

Primary Collaborators:
Dr. Nira Pollock, Beth Israel Deaconess
Dr. Donn Colby, Hospital for Tropical Diseases
Sarah McGray, PATH

• Study Objective: Assess agreement between the paper test and the standard venipuncture/laboratory assay

• Study Site: Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam

• Patient Population: HIV+ patients on ART with high HBV/HCV co-infection

• 700+ patient study underway
DFA has signed a commercial partnership with a major pharmaceutical company to develop hybrid LFT-specimen capture cards

**Desired capabilities:**
- At home LFT monitoring during clinical trials
- DBS, DPS for PK analysis post collection
- Format similar to DBS
- Remote data analysis

**Benefits of collaboration:**
- Accelerate clinical development
- DFA business model validation
- Leverage expertise of partner
- Distribution

**Dried Plasma Spot** on wax-patterned Ahlstrom 226 (DBS card)
Example 2: 3-D Immunoassay
3-D Immunoassay Concept

**Particle Layer** – Whatman + Dried Particles

**Dwell Layer** – Slightly hydrophobic channel to allow mixing of conjugate and antigen

**Capture Layer** – NC layer with bound Antibody

**Wash Layer** – Patterned Whatman paper

*Coated Sample Pad*  
*End Pad*  
*Coated Conjugate Pad*
3-D Immunoassay Concept

Antigen from sample is introduced to device & particles are released from paper

Antigen binds to particle during dwell

Antigen-particle complex binds to NC capture layer

Residual particles are washed down channel

Device peeled apart to read result
3-D Immunoassay

hCG (urine)

500 mIU/mL  50 mIU/mL  0 mIU/mL

Progesterone competitive assay (serum)

0 ng/mL  4 ng/mL

Malaria assays

Other tests for Malaria, Dengue Fever, and Sepsis are underway
“Diagnostic platforms that address the unmet needs of the resource-poor countries...will likely have substantial applications in profitable private markets of middle-income countries and in the developed world.” –BVGH

Diagnostics Innovation Map, 2010