Towards simple and instrument-free diagnostics…

…and their importance in global health

Workshop: Capillarity-based Microfluidics for Bioanalysis

October 1, 2011

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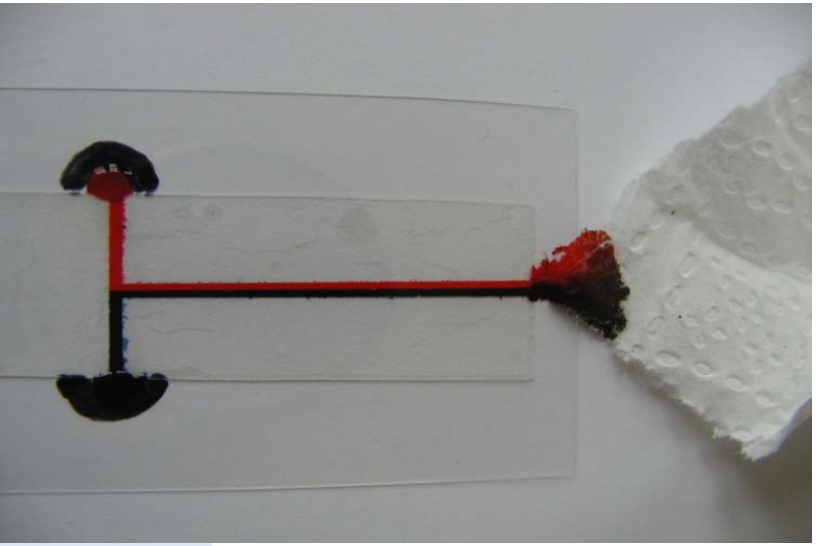
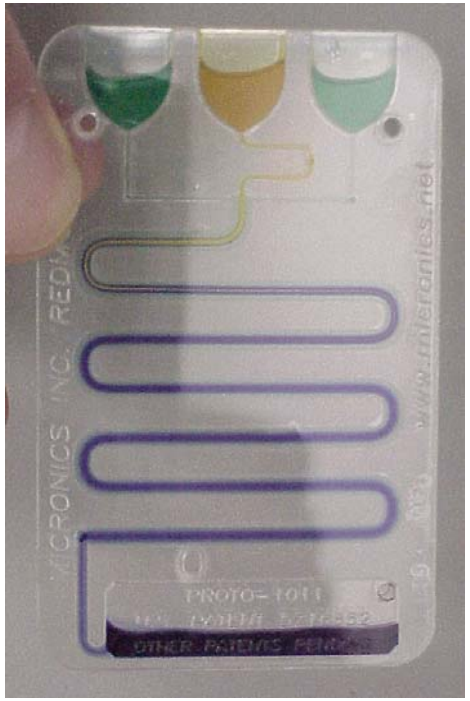
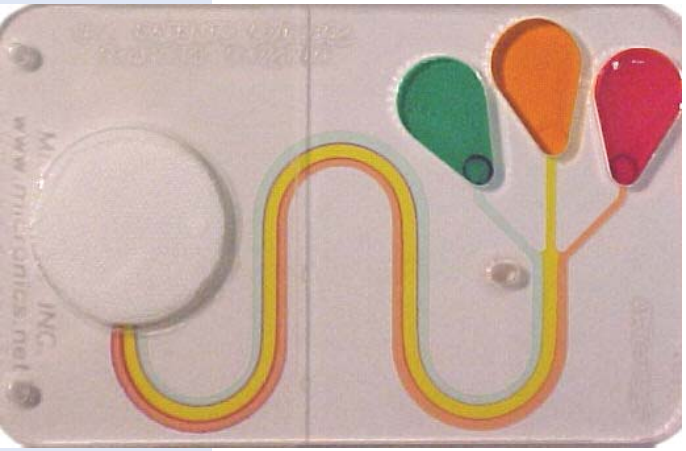
PATH Diagnostics Group
NIBIB Center for Point-of-Care Diagnostics
for Global Health (GHDx Center)

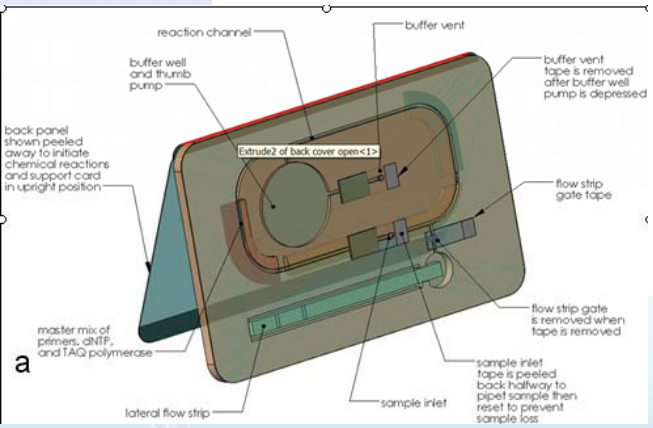
GHDx Center

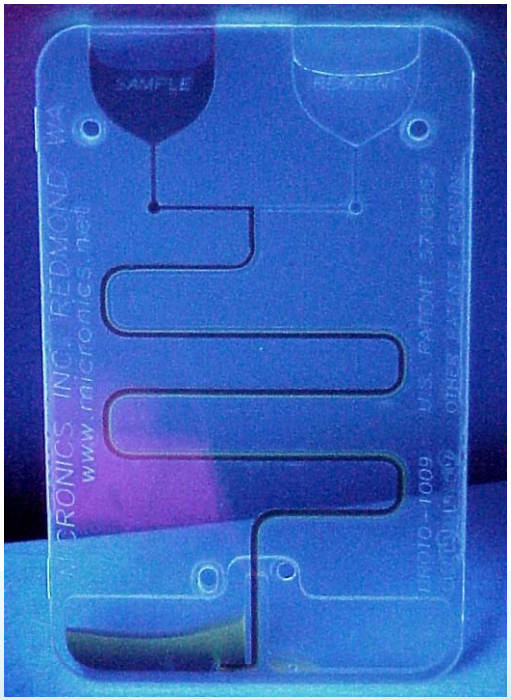
PATH
A catalyst for global health

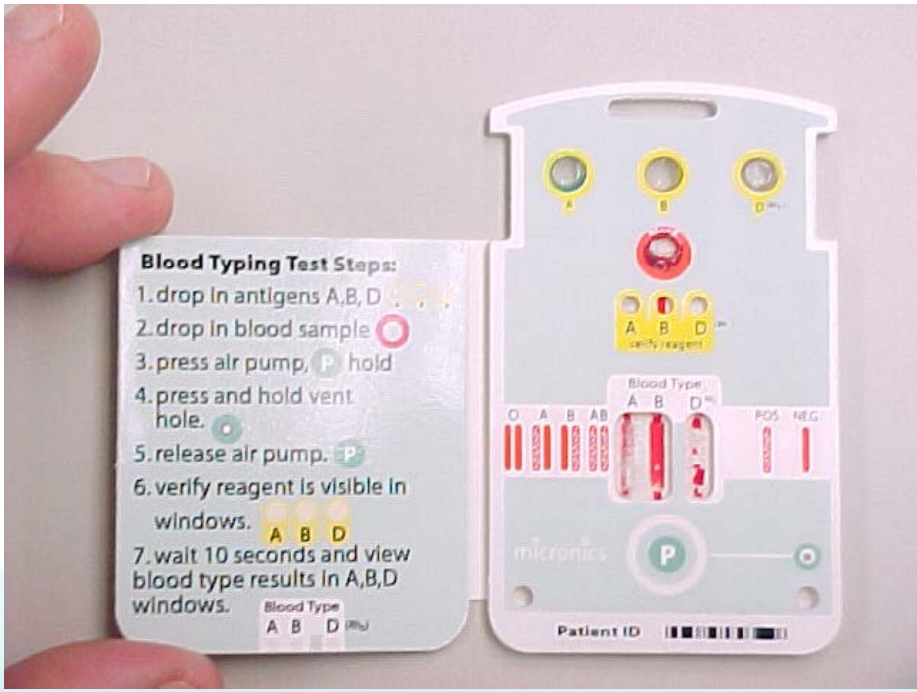







reaction channel
buffer vent
buffer well and thumb pump
buffer vent tape is removed after buffer well pump is depressed
back panel shown peeled away to initiate chemical reactions and support card in upright position
Extrude2 of back cover open<1>
flow strip gate tape
flow strip gate is removed when tape is removed
master mix of primers, dNTP, and TAQ polymerase
sample inlet tape is peeled back halfway to pipet sample then reset to prevent sample loss
sample inlet
lateral flow strip
a


SAMPLE
REAGENT


Blood Typing Test Steps:
1. drop in antigens A,B,D
2. drop in blood sample
3. press air pump, P hold
4. press and hold vent hole.
5. release air pump. P
6. verify reagent is visible in windows. A B D
7. wait 10 seconds and view blood type results in A,B,D windows.
Blood Type
A B D
Blood Type
A B D
O A B AB
POS NEG
micronics
P
Patient ID

- **This morning: *Towards simple and instrument-free diagnostics***
- Global health and the transformational potential of POC Dx in low resource developing country settings
- Why simple and "instrument-free"?
- Potential applications for paper microfluidics:
  - What is right and wrong with RDTs?
  - Isothermal NAATs on paper?
  - The chronic disease epidemic in LRS – quantitative assays on paper?
  - Integrating instrument-free assays with mHealth and Dx standards

This afternoon: *Simple diagnostics for complex markets*

- The PDP model of commercialization
- Sustainable commercialization
- Technology transfer and manufacturing models
- The potential and pitfalls of open source manufacturing

This talk

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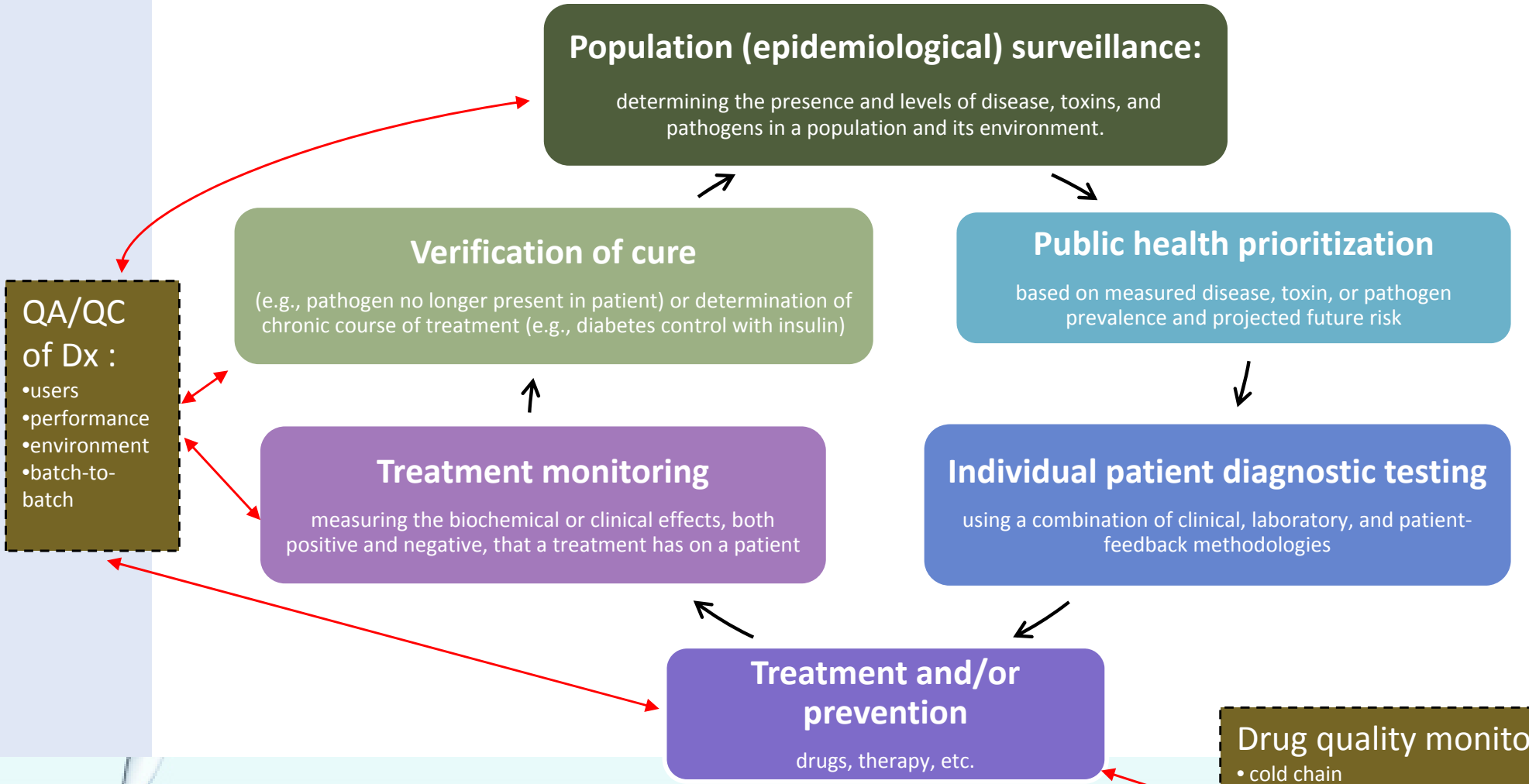
Diagnostics comprise 3-5% of health care spending, but influence 60-70% of health care decisions.

Source: The Lewin group. The value of diagnosis, 2009, commissioned by AdvamedDx; also stated in EDMA 2007 European Market Estimates

What if …we lived in a rational world: An information-based approach to health care



PATH: Program for Appropriate Technology in Health

History: Founded 1977

Focus: Developing technologies specifically for low resource settings

Number of staff: 800+ in Seattle and over 20 Country Offices

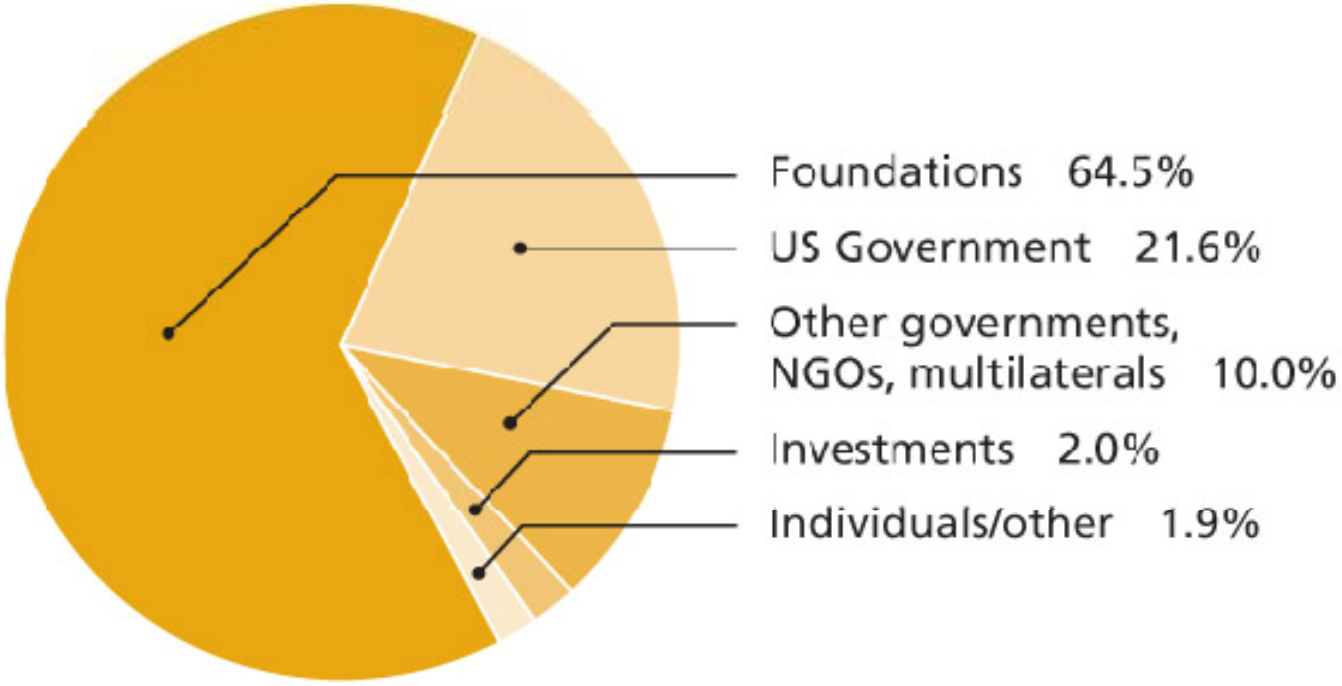
2010 budget (est.): US$ 300 million

Status: Private Non-profit

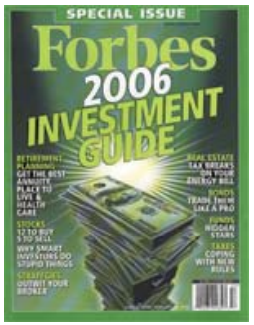
Fast Company
Social Capitalist Award
4 years running!

Four Star Charity
CHARITY NAVIGATOR

Charity Navigator highest rating

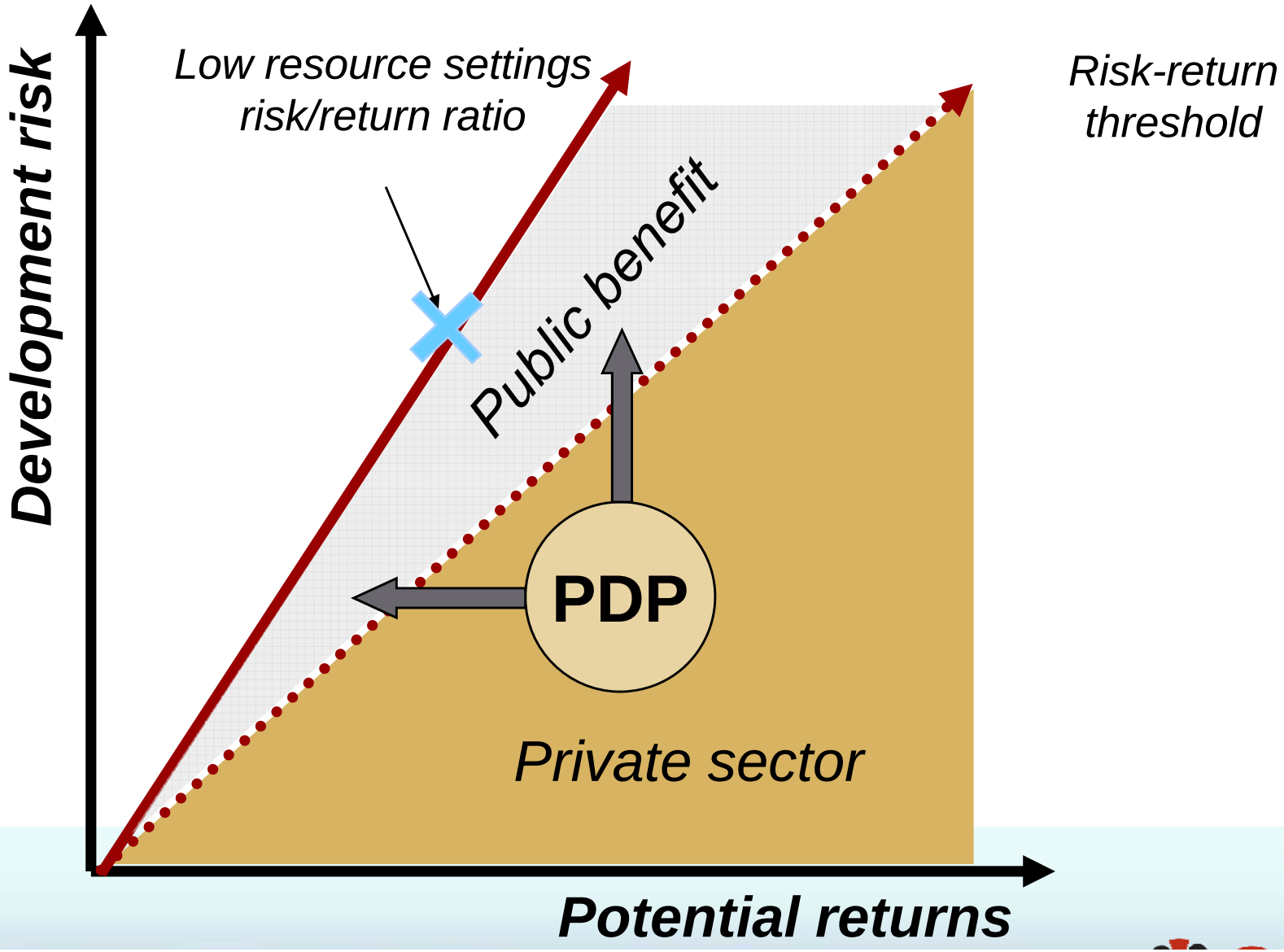


Amazon.com
Nonprofit Innovation Award



Forbes Top 200 Charities

PATH's role in product development partnerships



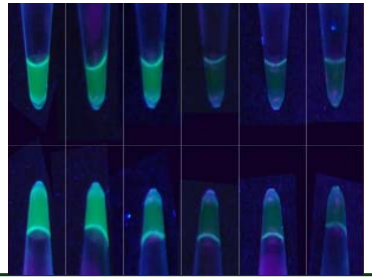
PDPs engage private sector to create public benefit by mitigating risks.

PATH Dx Group Capacity

0. Concept	1. Plan	2. R&D	3. Pilot	4. Transfer	5. Sustain
Needs ID & assessment	Discovery & feasibility	Development & prototyping	Pilot & evaluation	Introduction & deployment	Integration & sustainability

Market and User Needs

In-house R&D



Pilot Introduction

Training

Capacity Building

Dx R&D support and transfer

Due Diligence

Lab evaluation

Field evaluation

GHDx Center

Diagnostics Collaborators

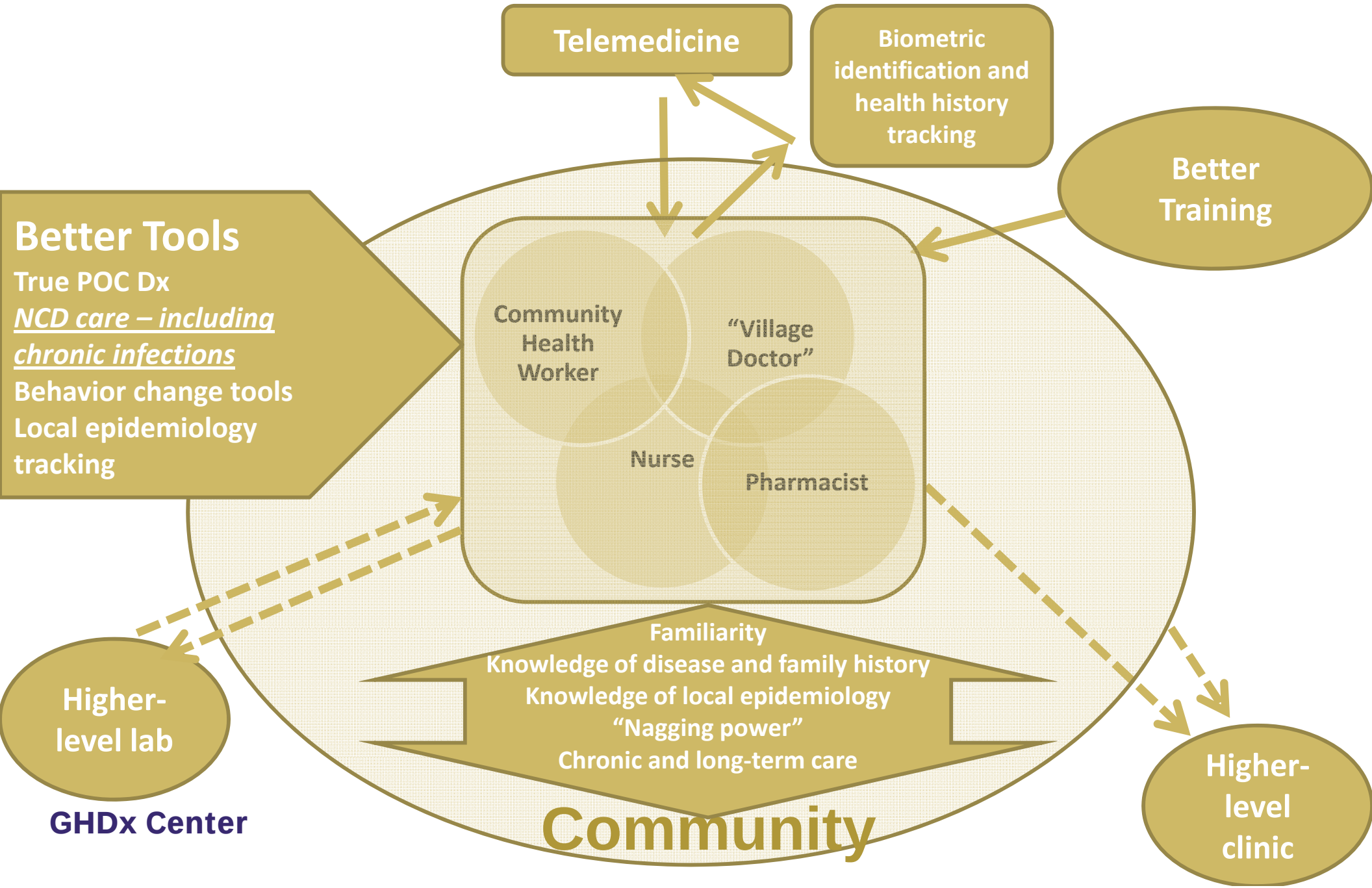
GHDx Center

POC Testing in developed countries:

- "Diagnostic testing that is performed near to or at the site of the patient care with the result leading to possible change in the care of the patient" (ISO22870).
- Key objective: generate a result quickly to influence treatment
- POC is designed as an *adjunct* to central lab testing, NOT as replacement
- With few (one) exception, POC has *not yet* been *transformational* for many patients or care givers

POC Diagnostics in developing countries

POC-Dx-enabled community-based care



Creating a POC based health system "from scratch" in developing countries

- The wireless phone vs. landline analogy
  - Can POC tests and telemedicine leapfrog developed-country-style central lab infrastructure?
  - Will central labs be niche or adjunct systems rather than the norm for diagnostic services?
- But:
  - Biosamples are harder to deal with than electrons
  - IT does not think in terms of QA and QC

This talk

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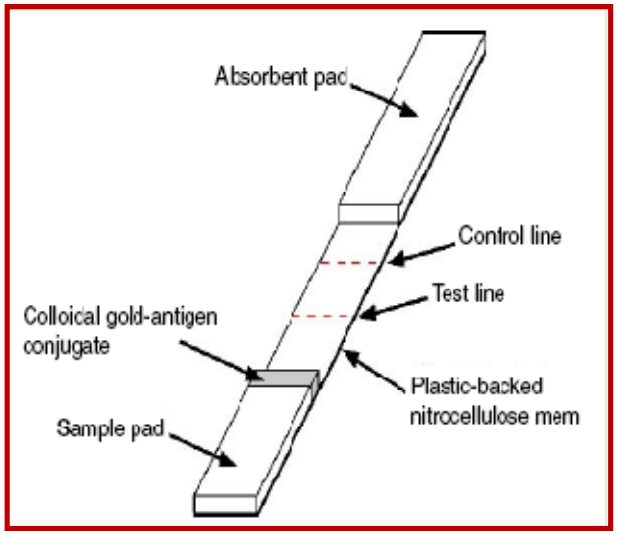
  - Integrating instrument-free assays with mHealth and Dx standards

The prototype simple and instrument free assay: Lateral Flow Strip Test

Inexpensive
Simple
Rapid
Convenient
Stable



Strip tests perform:

- *Sample prep*
- *Sample fluid movement*
- *Signal visualization*
- *Internal control*
- *Biowaste containment*

…at <US$1 and without an instrument!

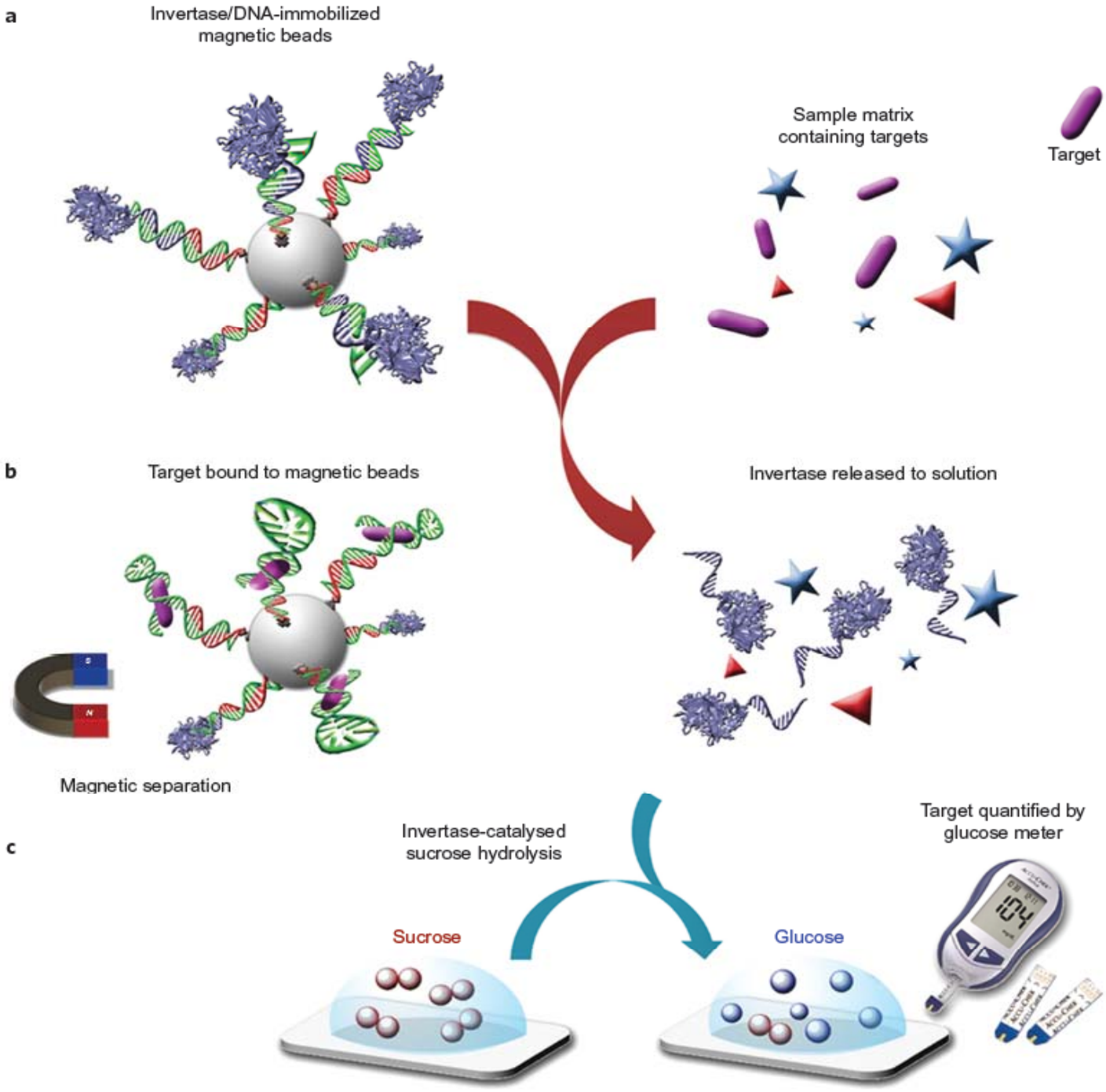
What is “instrument-free”?

- No equipment needed whatsoever?
  - How about pipettes, vials? DI water generator? A heater? Air conditioning? Fridge? Temperature-controlled shipping?
- Phones as detectors?
  - Cell phone cameras are powerful detectors and telemedicine communicators….but can they be FDA approved components of Dx devices?
  - Can *disposables contain all necessary information in or on them* (calibration, normalization, detector testing (e.g., identification through “virtual plug and play”?),… to allow <u>any</u> phone camera to provide a reliable, comparable result?

Why instrument-free?

- Logistics challenges for instrument supply, calibration, maintenance, repair
- Upfront cost deters Dx buyers (e.g., GeneXpert) – budgets may allow purchase of individual tests, but not capital equipment
- Tampering and theft of instrument
- Environmental factors lead to short life of instrument in LRS
- Training challenges for operation and maintenance of instrument

Other simple and ubiquitous detectors?



Using personal glucose meters and functional DNA sensors to quantify a variety of analytical targets, Y Xiang and Y Lu, *Nature Chem.*, 2011, DOI: 10.1038/nchem.1092

(Instrument-free) POC Testing for developing countries

Challenges

- Higher per-test cost in most cases
- Quality control risk
- Availability of even moderately trained health workers
- Test Procurement and Distribution
- Limits of performance
- Telemedicine can be supplement but can also disempower local capacity
- Sometimes central lab approach only possible solution (no POC test available)

Advantages

- Lower start-up cost
- More flexible innovation
- Customizable solutions
- Empowering local health care providers
- Minimal infrastructure necessary
- Rapid turnaround of test results and start of treatment
- Sometimes POC only possible solution (limited access to, slow response from central lab)

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Lateral Flow Strip Test – drawbacks

- Lacking in sensitivity and specificity
- Difficult to provide quantitative result
- User errors; interpretation needed
- Low barrier to manufacturing leads to QC issues
- Multiplexing difficult
- Complex, multistep RDTs difficult

→ Can paper microfluidics address this?

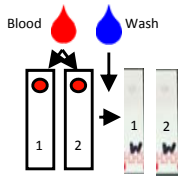
NAATs on paper? Maybe.
For now– focus on component improvement

1. Use amplification chemistry that can use 'dirty' DNA – no/little sample prep
2. Simpler, isothermal amplification; heating with exothermic reaction
3. Use low cost, field friendly materials for disposable
4. Interchangeable modules for different niches

Same goal: simplicity of strip test, sensitivity of PCR

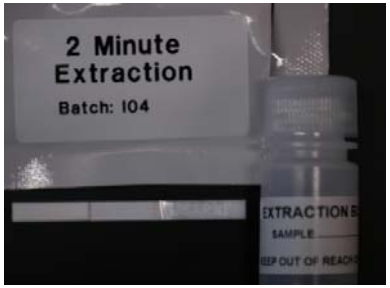
Strategies for Nucleic Acid Amplification at the Point-of-Care

Sample Preparation | **Target Amplification** | **Heating/ Instrumentation** | **Signal Detection**

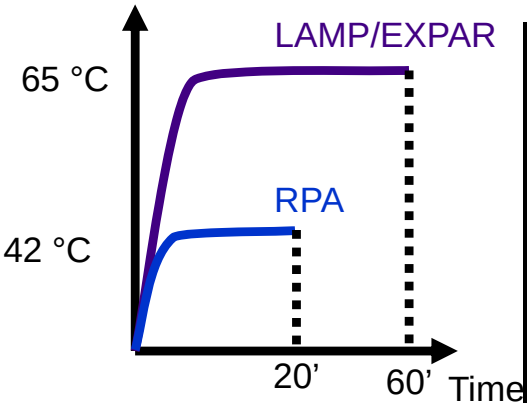


DNA capture card

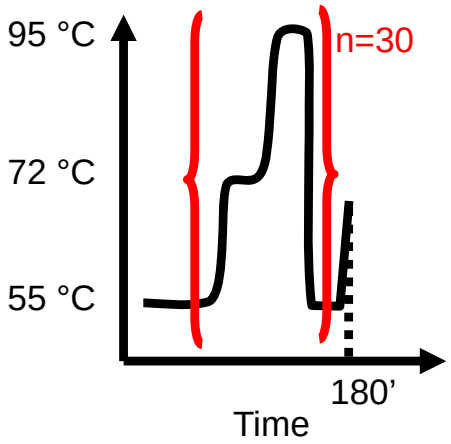
Nucleic acid extraction kit



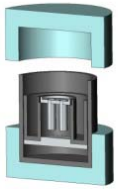
LF nucleic acid extraction kit



PCR



Isothermal chemistries

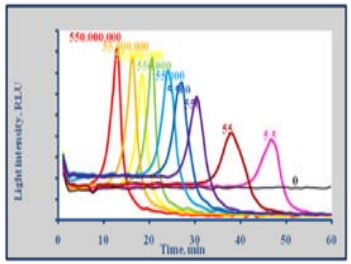


Non-instrumented nucleic acid assay (NINA)

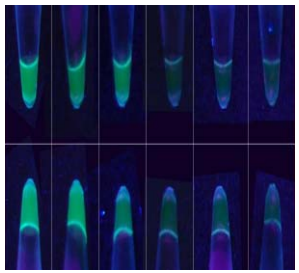
Qualitative instrumentation

Quantitative instrumentation

Lateral flow amplicon detection



Instrumented detection

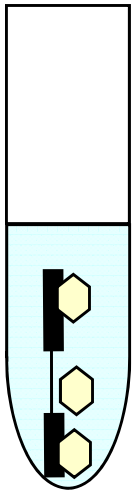


Manual detection

GHDx Center

Sample Preparation

PATH Nucleic acid extraction kit and card



vs.

Qiagen Qiacube automated sample prep

NINA Heating

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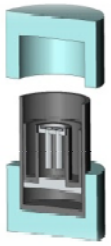

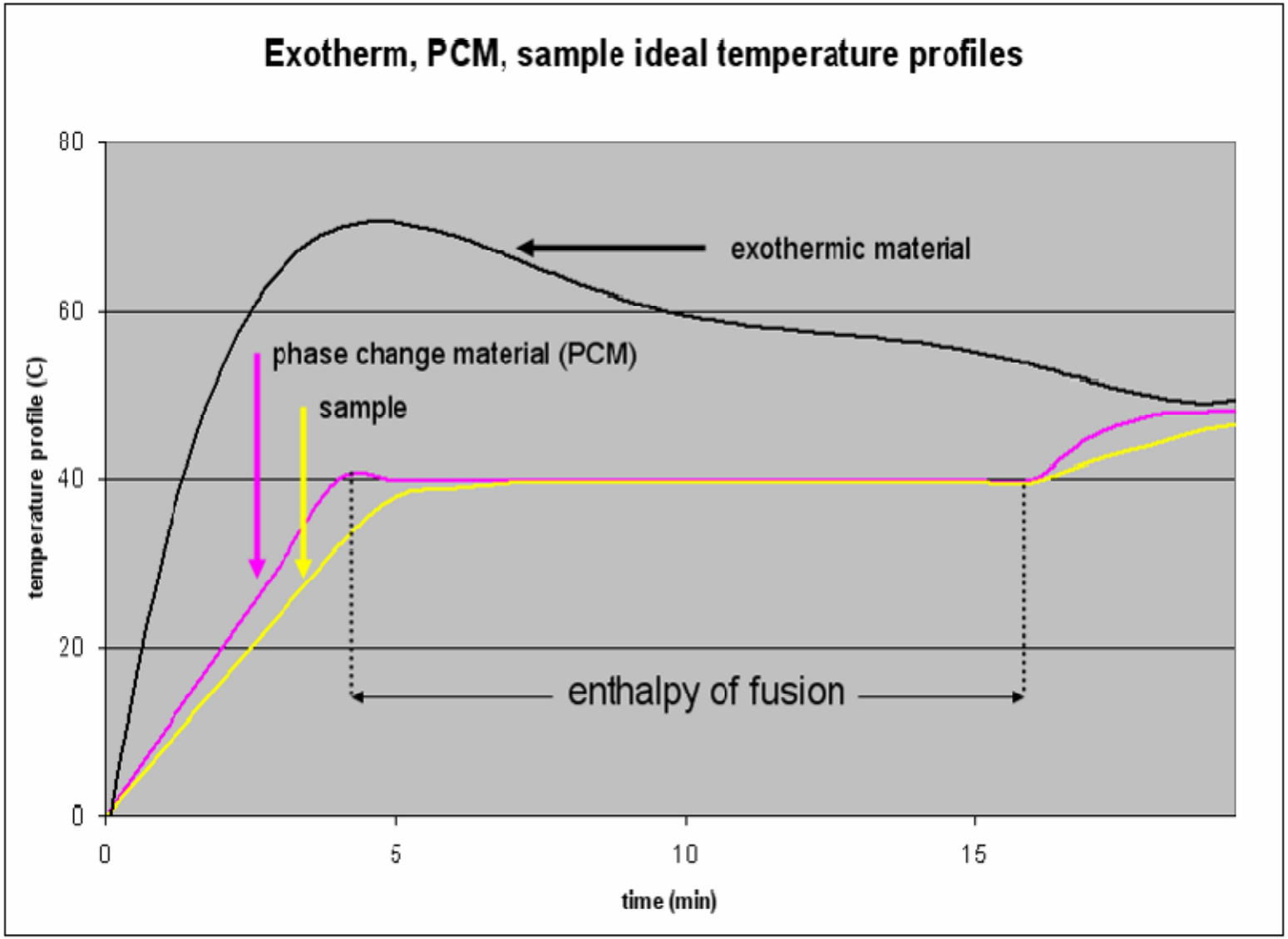

NINA Heating

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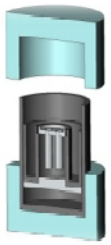
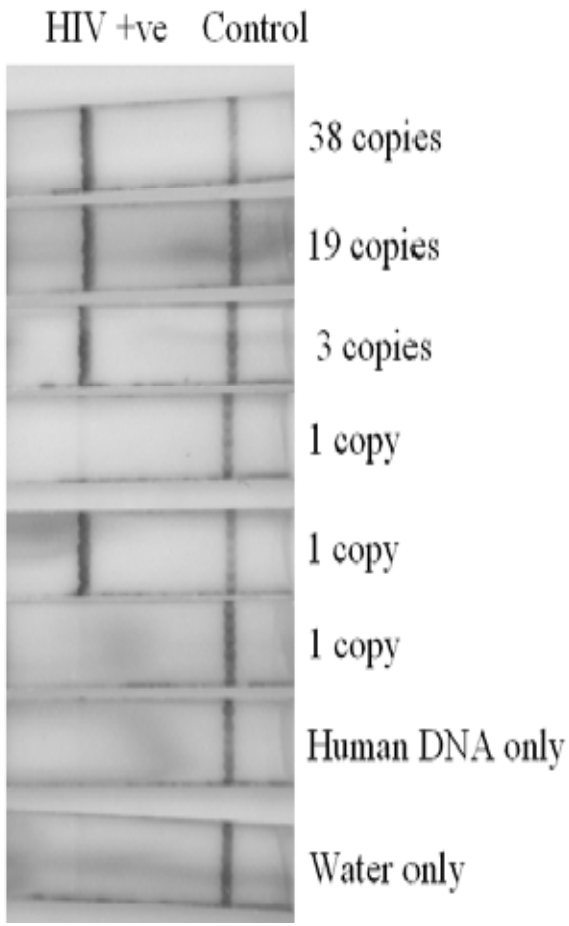


Visual and LFS Detection of NA amp

gDNA ng/µL	~ Copy #	NINA	PCR	NINA	PCR
0.1	5000				
0.01	500				
0.001	50				
0.0001	5				
2.00E-05	1				
NTC	0				



Detection: Turbidity Fluorescence NA LFS

Chronic disease (NCD) epidemic in LRS

- Diabetes now bigger cause of morbidity and mortality than infectious diseases in LRS

- Infectious disease: presence or absence most critical
- For NCD: quantitation paramount to guide treatment

- Chronic disease requires continued testing – low cost, ease of use, POC availability very important

- Some IDs are like NCDs: HIV, TB, some NTDs

Countries with the most persons with diabetes, 2010 updated

	Country	2010 Millions
1	India	100
2	China	100
3	USA	26.8
4	Russian Federation	9.6
5	Brazil	7.6
6	Germany	7.5
7	Pakistan	7.1
8	Japan	7.1
9	Indonesia	7.0
10	Mexico	6.8

Source: Jonathan Brown, IDF

Diabetes Mellitus (DM) and Gestational Diabetes (GDM)

- GDM appears to cause DM in offspring…
- ….especially in future mothers
- Famine causes DM, hugely…especially adequate food becomes available afterwards
- Low birth weight (regardless of cause!) causes DM…especially adequate food becomes available afterwards
- Obesity and lifestyle are much more loosely correlated with DM and GDM in developing countries – other factors at play

Current diabetes screening:

Diabetes Mellitus (DM):

- Random blood glucose testing: low cost, but very inexact
- Fasting glucose testing: requires patient preparation and time
- Urine glucose strip: low cost, can not find borderline cases (may be good primary screen)
- HbA1c: needs instrument, currently pricey on a per test basis

Gestational Diabetes Mellitus (GDM):

- OGCT – requires fasting, a baseline blood glucose test, a glucose challenge, and at least one additional blood glucose test

Some needs are already identified:

- **Diabetes Mellitus Type 2 (DM):** low-cost, non-invasive screening
- **Gestational Diabetes (GDM)**: low-cost screening that does not require fasting or prolonged clinic visit

1. Can paper microfluidics provide for a multiplexed, fairly quantitative assay to screen for diabetes, and monitor diabetes treatment efficacy – (blood glucose, HbA1c, creatinine, glycated albumin, fructosamine, advanced glycation end products?
2. Can paper microfluidics create a cheaper glucose test?

Instrument-free POC Dx, mHealth, and Standards

- Dx instruments can be easily connected to LIS and telemedicine
- Results from instrument-free tests should also be recorded and reported
- Cell phones for recording and reporting (different from detection)?
- Making paper microfluidics compatible with ports on standardized Dx platforms?

Detour: The BMGF GC Diagnostics Standards Initiative

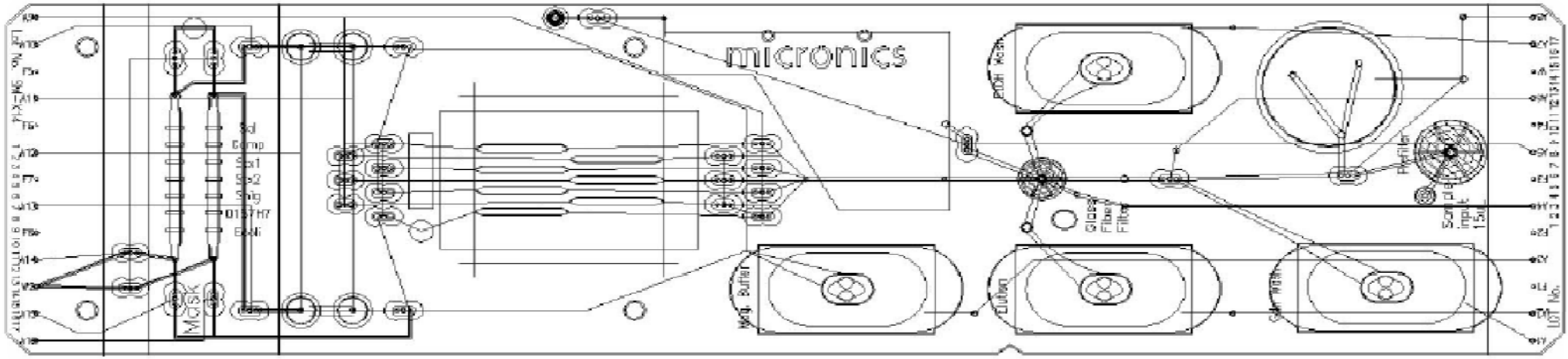
- $30-50 M to be invested by BMGH and GC Canada in next generation diagnostics
- Initiative to set standards for future diagnostics by any participating manufacturers
- Idea: Create open standards and installed base, and let anyone develop and commercialize assays and components that fit together
- Standards can:
  - Drive adoption through installed base of instruments
  - Create “quality seal of approval“ in the absence of unified regulatory system
  - Focus manufacturers on needs of LRS

s

No Diagnostics Interface Standards?

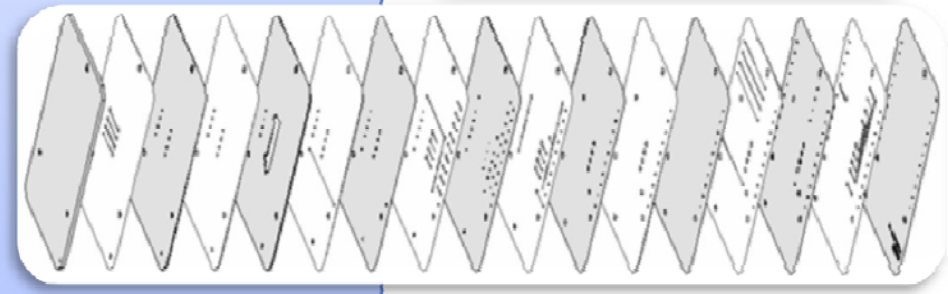
- Many intra-company closed interface standards:
  - iSTAT
  - Large lab chemistry analyzers
  - Other POC chemistry analyzers
- ICS is "sort of" standard – not *interoperability*, but *usability* standard
- Should paper microfluidics have standardization element?

GCGH Round 1 - DxBox/DEC Experience[s]



- simple instrument, complex disposable
- all NAAT functions, reagents, and heterogeneous components integrated on laminate-based card
- sample in/result out, but:
  - expensive disposable
  - failure-prone, complex manufacturing
  - Flexible in principle

  (different cards for different purposes, but design complexity makes development of new cards difficult)



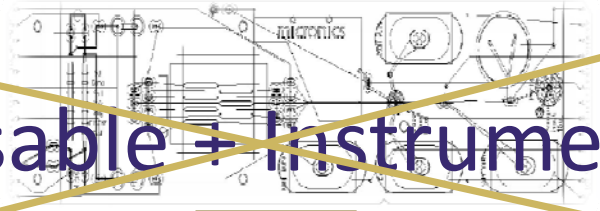
Instrument-based Diagnostics: Complexity - where should it be placed?

	INSTRUMENT	DISPOSABLE	USER
Initial system cost	high	low	low
Per test cost	medium	high	low
Training requirement	medium	low	high
Service requirement	high	low	low
Lab requirement	high	low	medium
QC complexity	medium	low	high
Performance	high	Low-med	Low-med

Lesson from DxBox, GeneXpert, etc?

For multi-target Dx device:

~~Disposable + Instrument~~



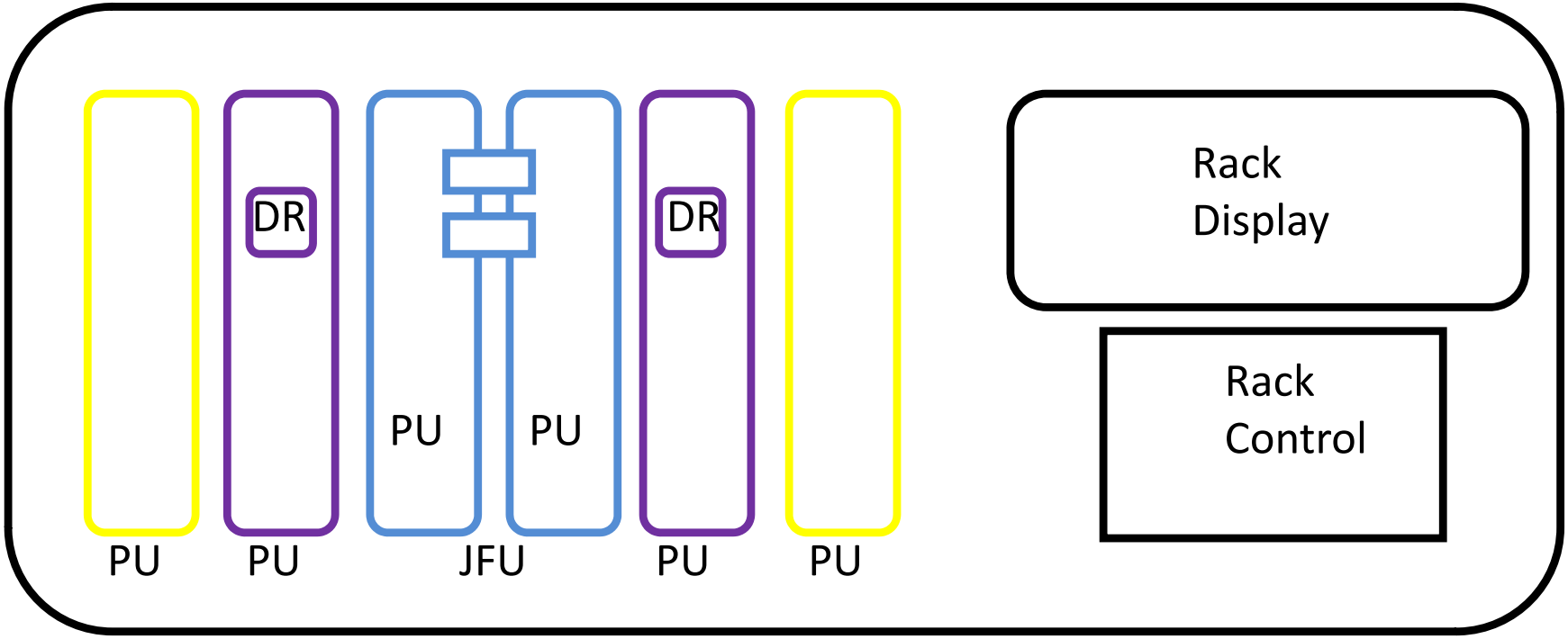
Disposable + Modules + Rack?

(shared complexity)

Straw Man - Integrated Device Schema

- *"Rack" – "Platform Unit" – "Disposable"*
- Disposables handle chemistry and fluids
- Platform units handle mechanics, optics, and electronics of assay
- Rack handles operating system, power, and communications
- Two-level device customization through selection and combination of platform units and disposables

Straw Man - Device Schematic:



Black: Rack and Rack controls

Purple: Platform Unit (PU) with Disposable Receptacle (DR)

Yellow: PU without DR

Blue: Joint Functional Unit (JFU)

Red: Stack of disposables to be inserted in one of the DRs; comprise sample receptacle and assay chemistry

Straw Man - Sample Format

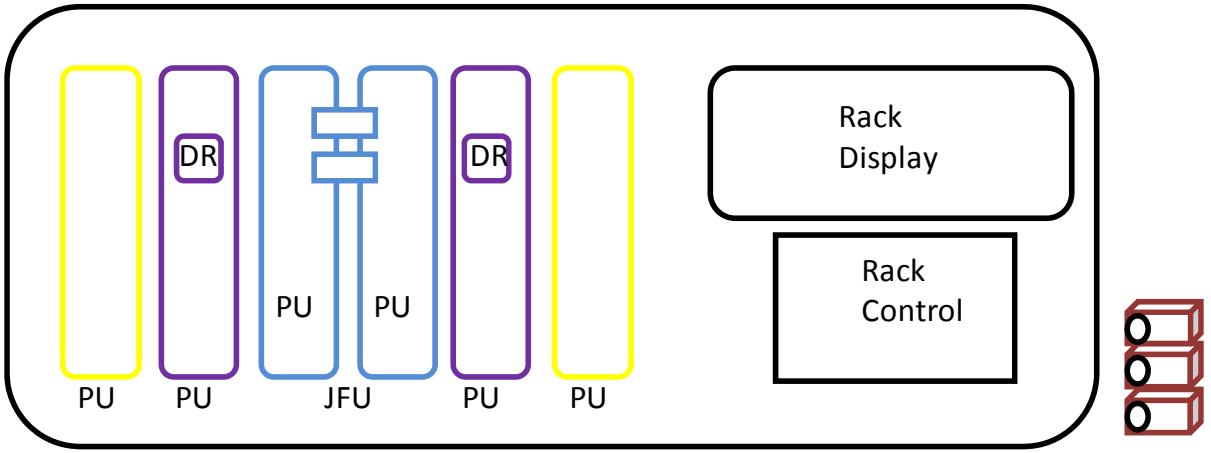
- Defined by the platform unit that handles that particular assay.
- All standard sample formats should be accommodated in principle – blood, serum, saliva, sputum, eluent from cervical, vaginal, anal swabs, etc.
- The “rack” will not accept samples – individual units will.

Straw Man - Levels of Interface Standards

- Level 3 – Disposable **X** Platform Unit (PU)
- Level 2 – Rack **X** Platform Unit (PU)
- Level 1 – PU **X** PU to form JFU
- Level 0 – Rack **X** World)

- Use existing standards (IEEE etc.) where possible
- all levels of interface standards should ideally be **open**
- a developer can design a PU or JFU for the “rack” that can run a number of specific assay disposables, or an assay disposable for another manufacturer’s PU.

Is the BMGF standards process relevant for instrument-free (paper) Dx?



- Could a standardized platform have a portal for “less standardized” non-instrumented assays (RDTs and paper microfluidics?
- I.e., a camera? Something else?
- Installed base as driver for adoption even for paper microfluidics?
- The broken regulatory system in LRS – can standards help?

Thank you!