PORTABLE MOLECULAR DIAGNOSIS OF HPV

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Cervical Cancer and HPV

HPV is the causative agent in most cervical cancers.

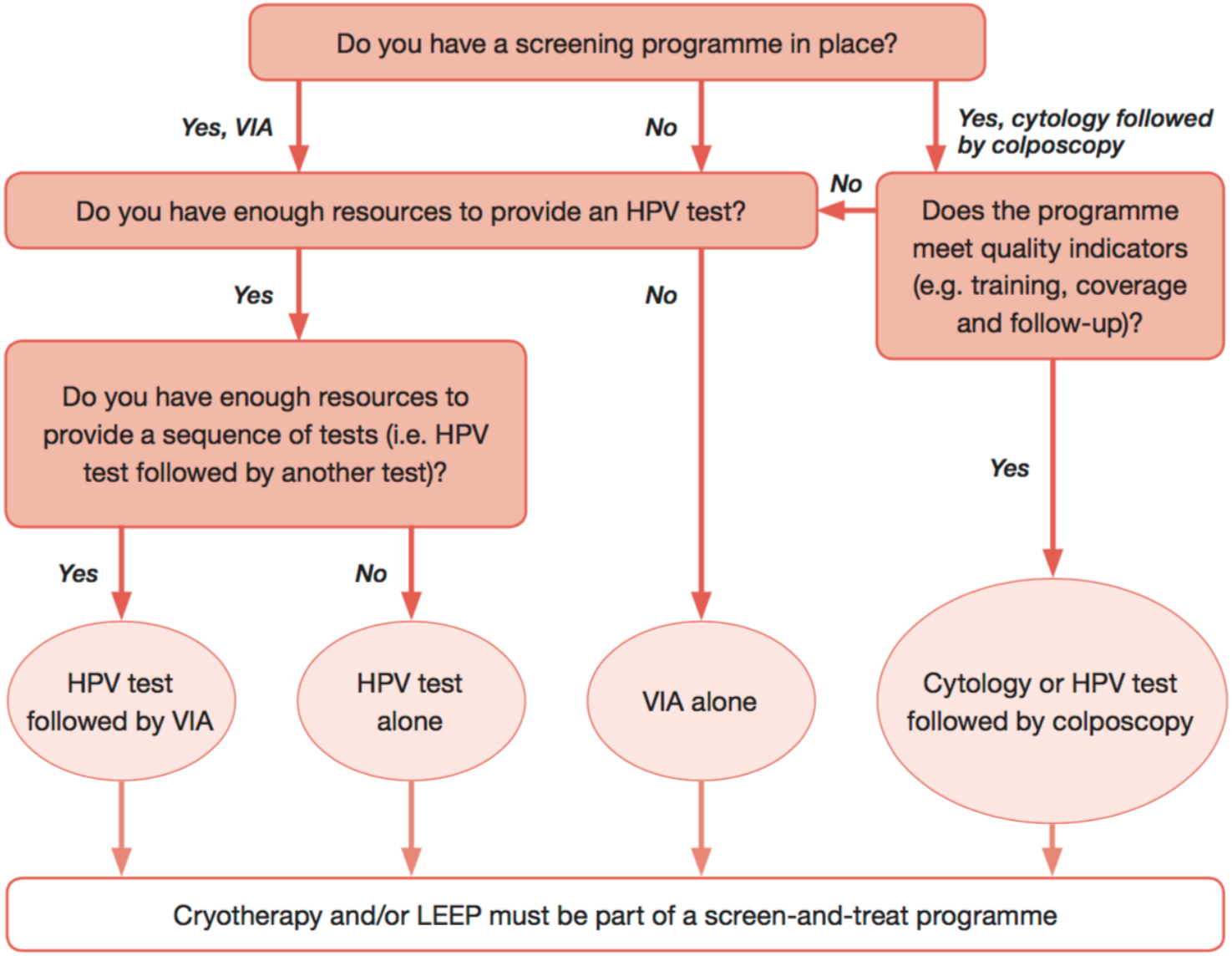
Cervical cancer is the fourth most common cancer in women worldwide and kills 266,000 women per year.

Approximately 87% of these cases are in developing countries, where there are limited resources for screening.

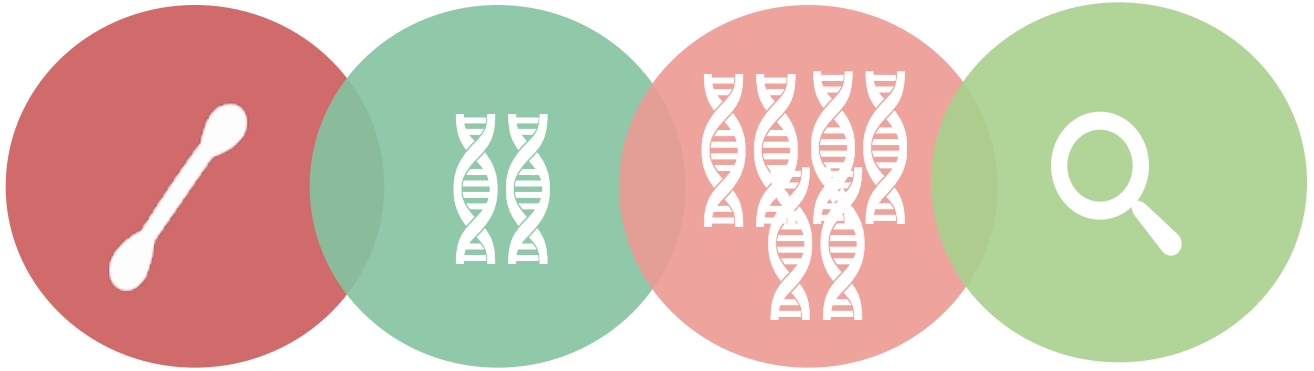
When diagnosed early and accurately, cervical cancer has a high 5 year survival rate, emphasizing the value of early detection.

WHO Guidelines for Cervical Cancer Screening

What happens if there's a positive test? Where does this fit in to the treatment chain?



Components of a Portable Molecular Diagnostic

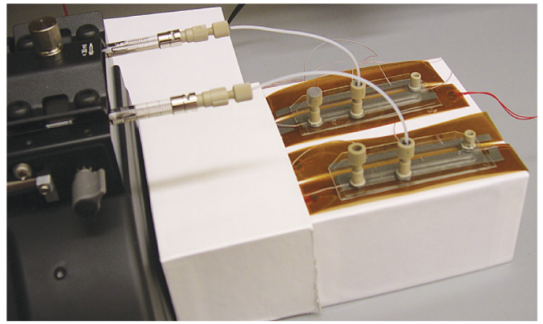


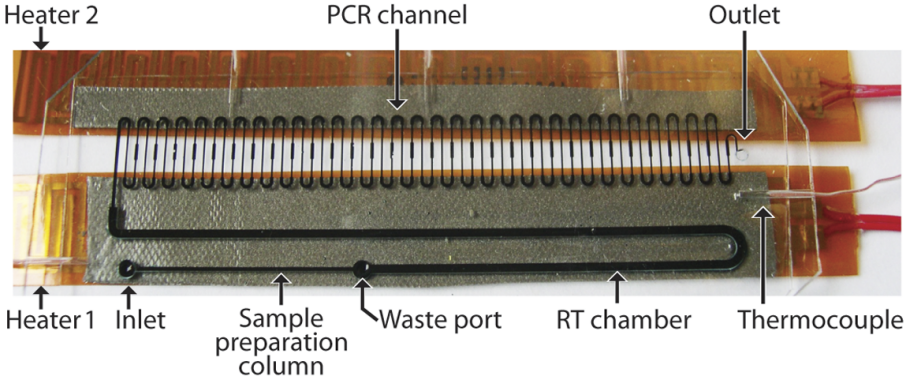
Sample Collection

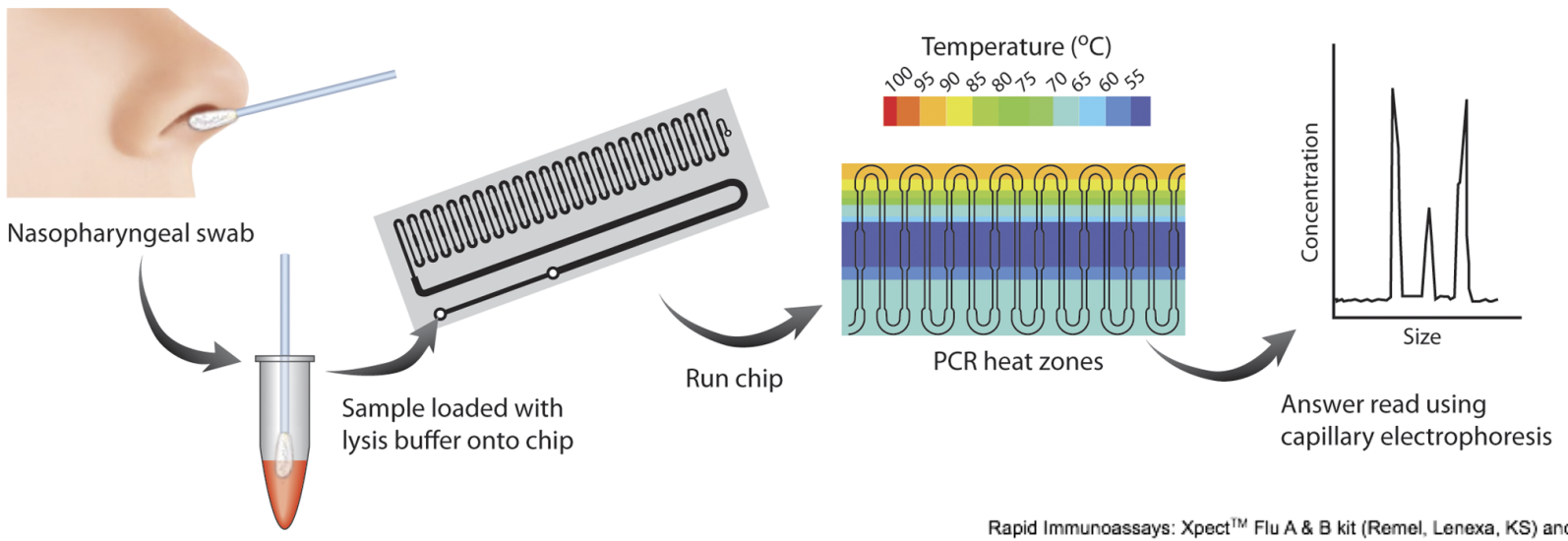
Isolate nucleic acids

Amplify Nucleic Acids

Readout







- 146 NPA and NPS samples were collected during 2008-2010 at Boston Medical Center (BMC) Beth Israel Deaconess Medical Center (BIDMC).
- Patients presenting with one or more of: fever, cough, sore throat, myalgia, nasal congestion, headache, malaise, or diarrhea.
- 12 months to 70 years.
- RT-PCR Chip for INFLA

Microfluidic Assay	Benchtop RT-PCR			
(n=146)	positive	negative		
positive	70	0	100% *(94%,100%)*	PPV
negative	3	73	96% *(88%,98%)*	NPV
	96% *(89%,99%)*	100% *(95%,100%)*		
	Sensitivity	Specificity		

Rapid Immunoassays: Xpect™ Flu A & B kit (Remel, Lenexa, KS) and the BinaxNOW™ Influenza A & B kit (Inverness Medical, Princeton, NJ)

Rapid Immunoassays	Benchtop RT-PCR			
(n=119)	positive	negative		
positive	33	1	97% *(85%,100%)*	PPV
negative	34	51	60% *(49%,70%)*	NPV
	49% *(38%,61%)*	98% *(90%,100%)*		
	Sensitivity	Specificity		

Cao Q et al., *PLoS ONE* 7(3): e33176. (2012)

Sample	Infectious Agent	LOD	Detection Method
Nasowash/ Nasoswabs	*Influenza A*	~10^4 copies/ml ~10^2 – 10^3 pfu/ml	PCR[1]
	VSV (Ebola psuedotypes)	10^4 pfu/ml	Interference spectroscopy
Urine	*E. coli*	10 CFU/ml	PCR Off chip[2]
Blood	*E. coli* *B. subtilis* *E. faecalis* *HIV*	10^2 CFU/ml 10^3 CFU/ml 10^4 CFU/ml 10^2 copies/ml	PCR Off chip[3,4], SERS PCR, bDNA[7]
Stool	*C. difficile*	0.01 pg DNA Human samples	PCR[5] and HDA On chip[6]
Vaginal Swabs	*CT/NG*	Ongoing work	HDA on chip

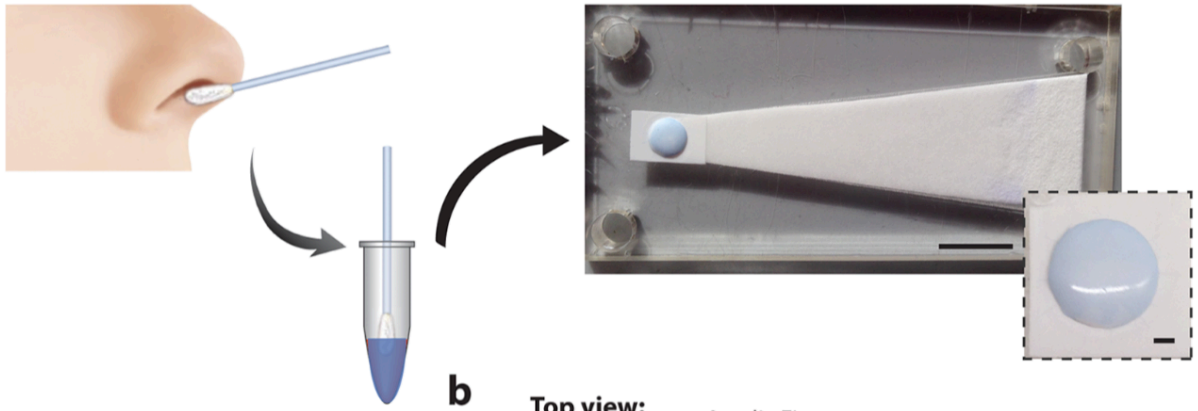
[1]Cao Q et al., *PLoS ONE* 7(3): e33176. doi:10.1371/journal.pone.0033176 (2012)
[2]Kulinski, M.D, et al., *Biomedical Microdevices*, 11(3), pp.671-678, (2009).
[3]M. Mahalanabis, et al., *Lab on a Chip*, 19, pp. 2811-2817 (2009).
[4]Sauer-Budge, A.F., et al., *Lab on a Chip* **9**, 2803 (2009).
[5]Gillers, S., et al., *J Microbiol Methods,* 78(2):203-7 (2009).
[6]Huang S, et al. (2013). *PLoS ONE* 8(3): e60059. doi:10.1371/journal.pone.0060059
[7] Byrnes, et al. (2013). ***Anal. Methods***, 2013,**5**, 3177-3184

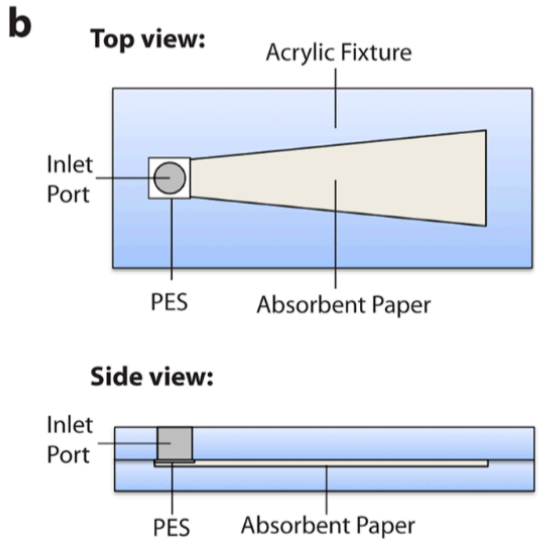
Design for POC – Design for Minimal Instrumentation

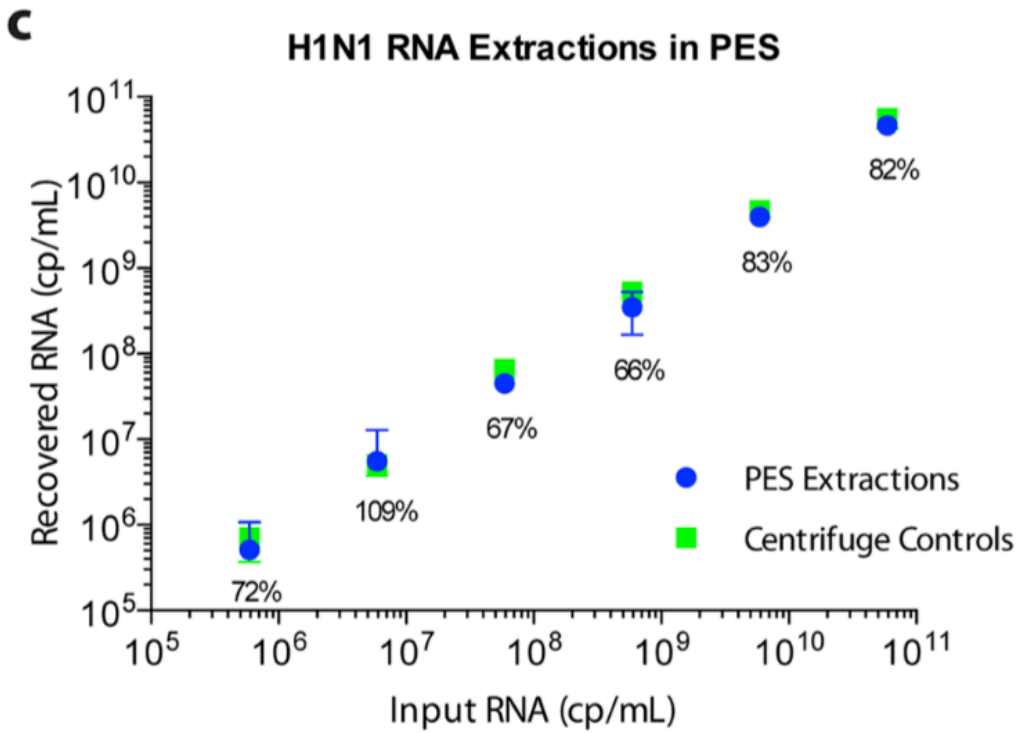
Goal: Get a molecular test to a remote clinic.

- Size
- Shelf life
- Passive flow
- Heat with minimal/no instrumentation
- Intuitive readout without instrumentation

Nucleic Acid Isolation

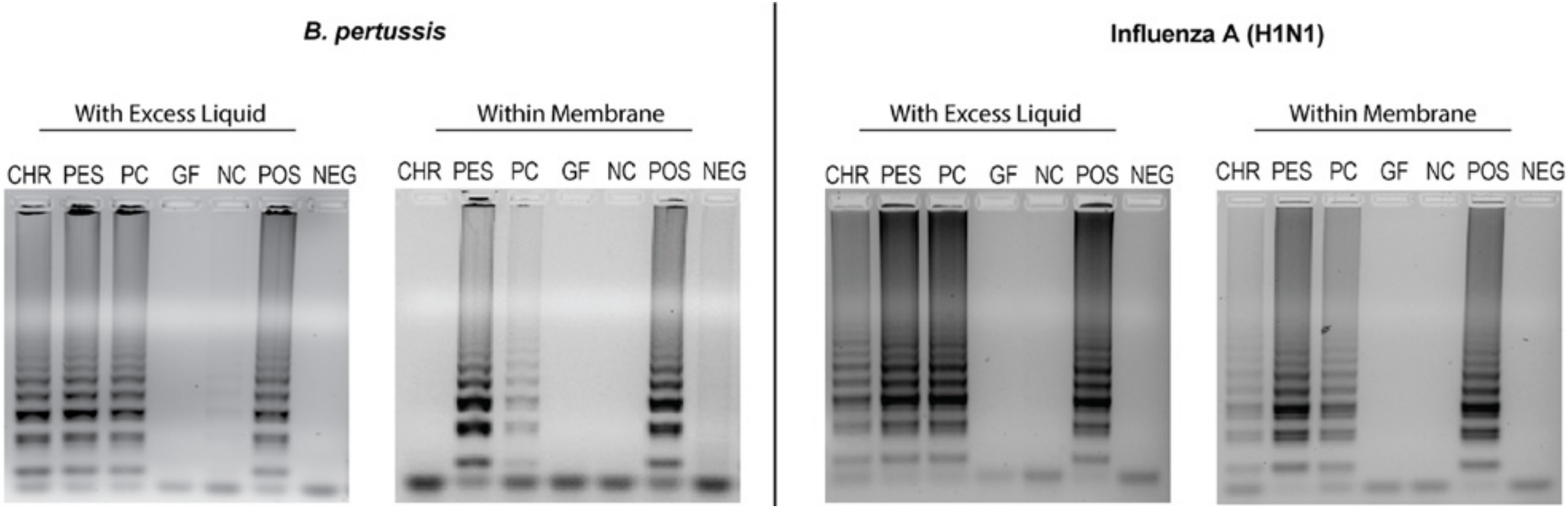






Rodriguez et al., Anal. Chem., **2015**, *87* (15), pp 7872–7879

Validation of Amplifiable NA Analysis of LAMP in Different Papers

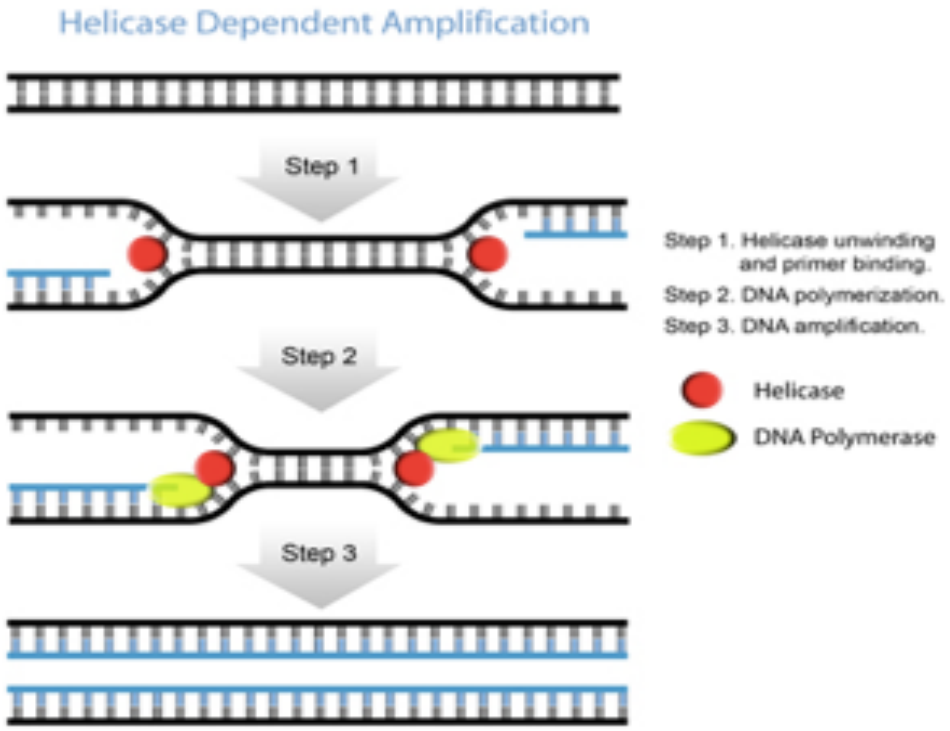


Linnes *JC, Rodriguez NM, Liu L,* ***Klapperich*** *CM, Biomed Microdevices. 2016 Apr;18(2):30.*

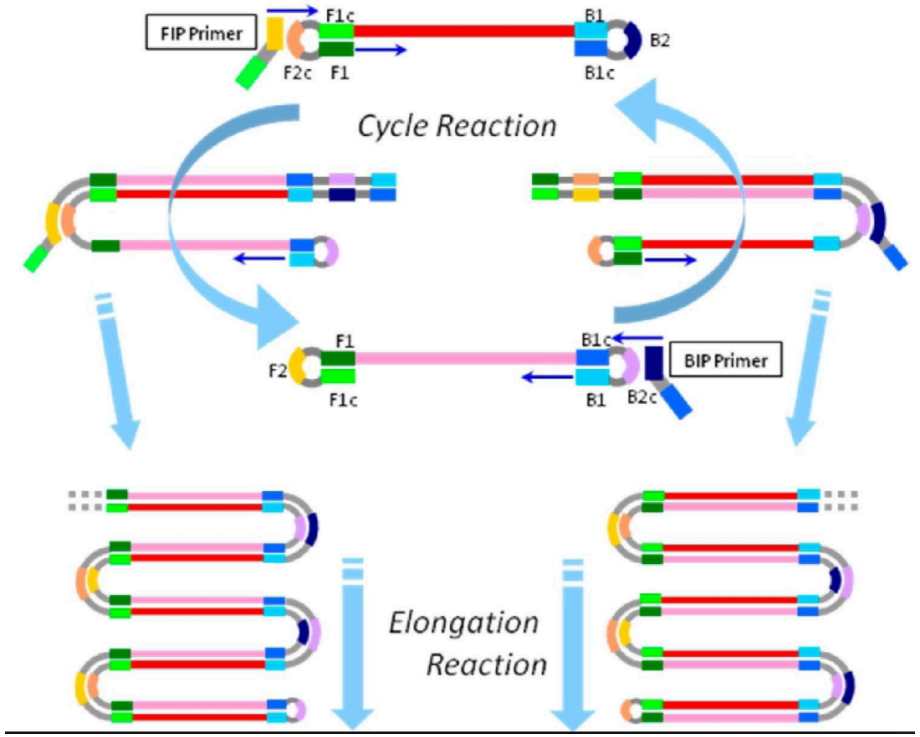
Nucleic Acid Amplification

Isothermal Techniques

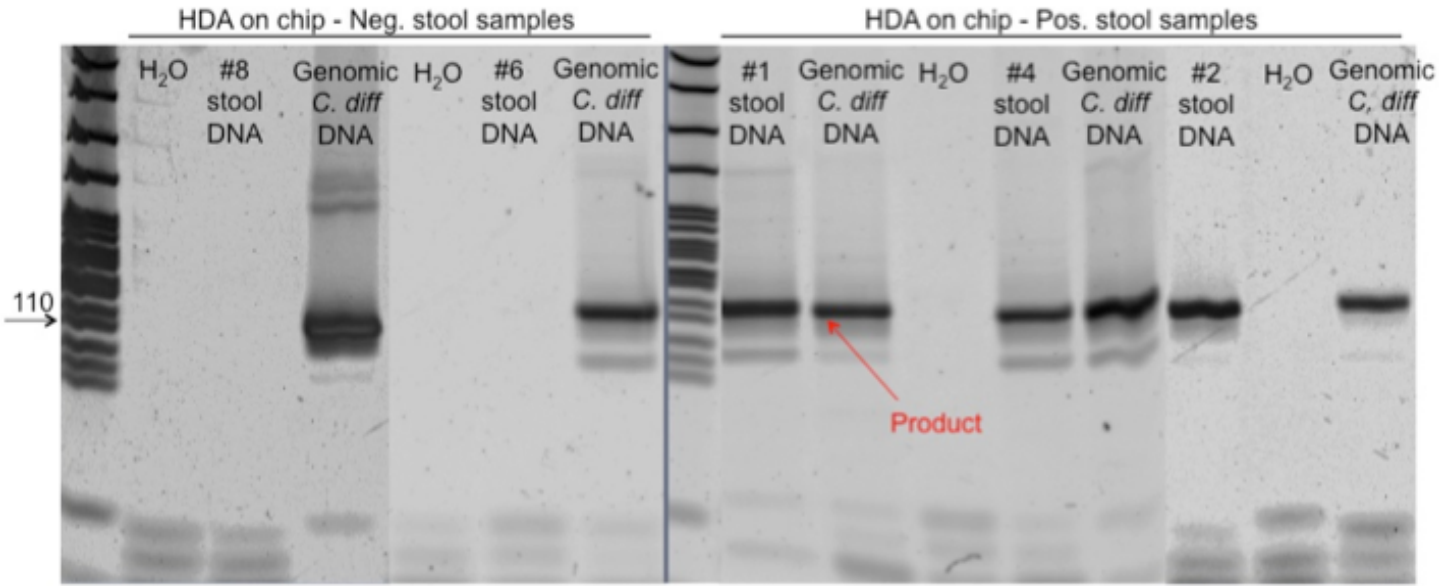
HDA

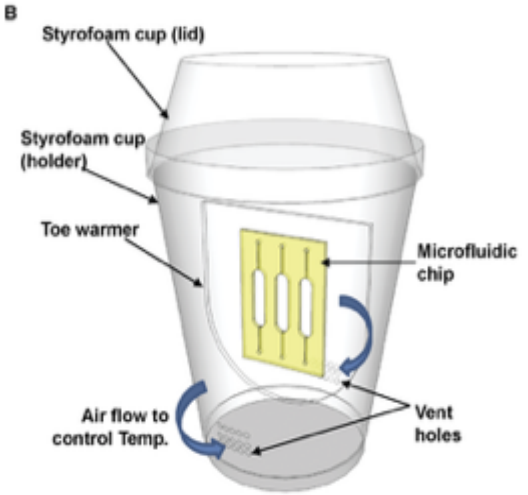


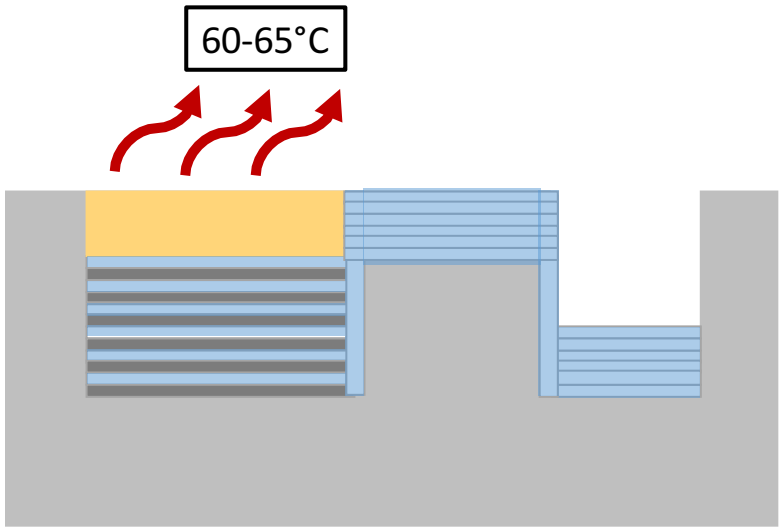
LAMP

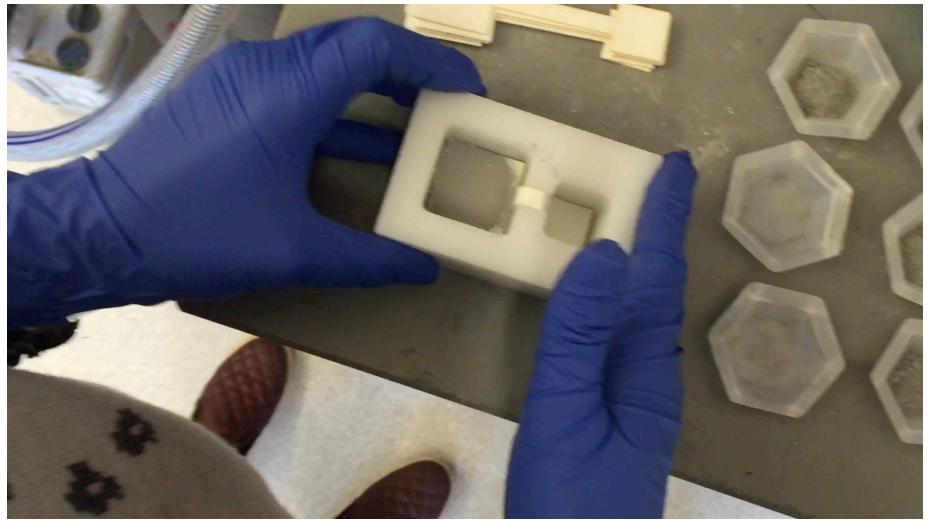


DNA Extracted from Stool, Amplified without Instrumentation





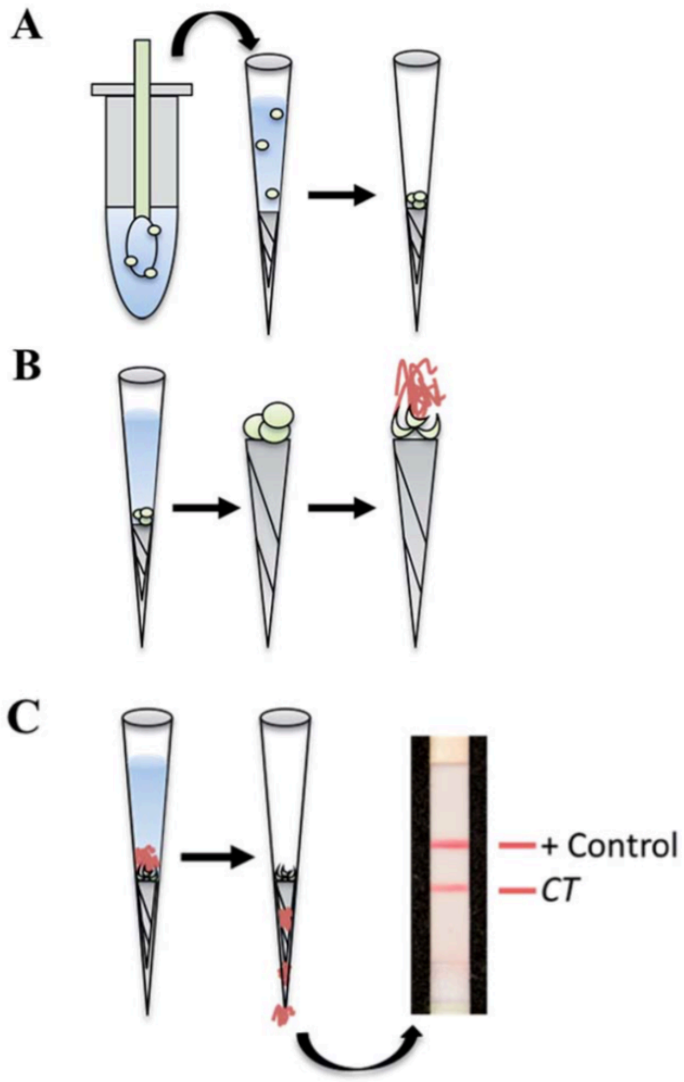


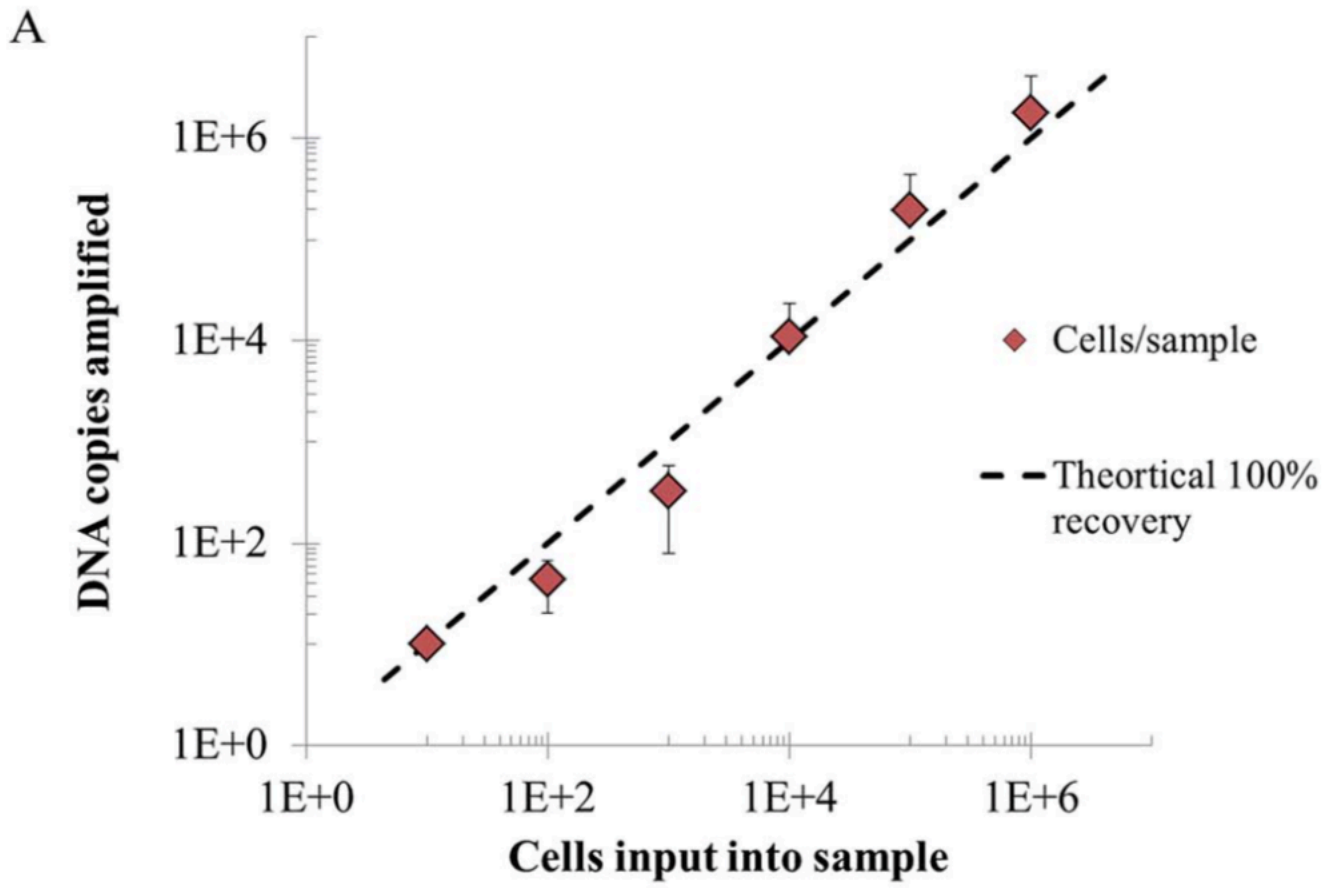


Huang S, et al. (2013), PLoS ONE 8(3): e60059.

Lysis, Isolation and Amplification on Paper

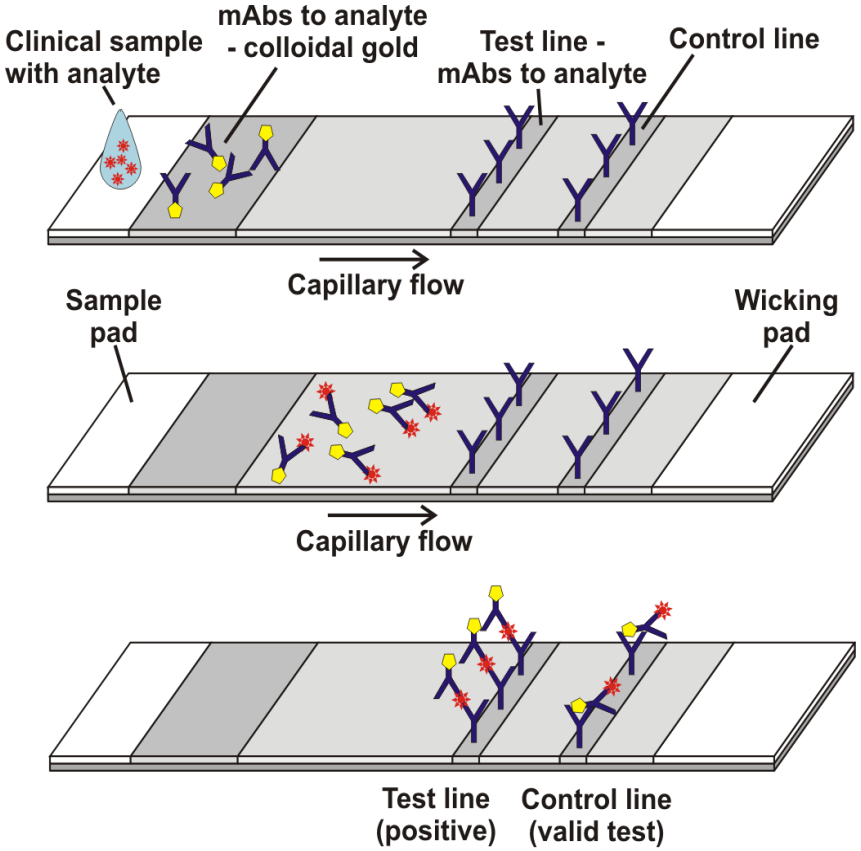
Chlamydia Cells (DNA)

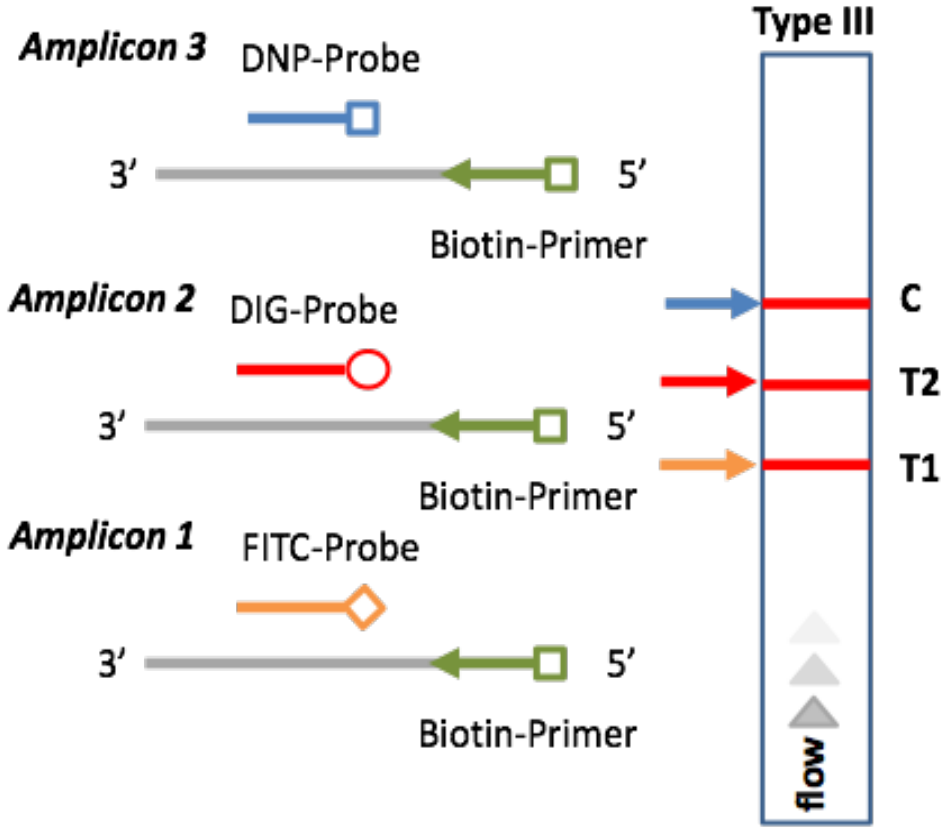




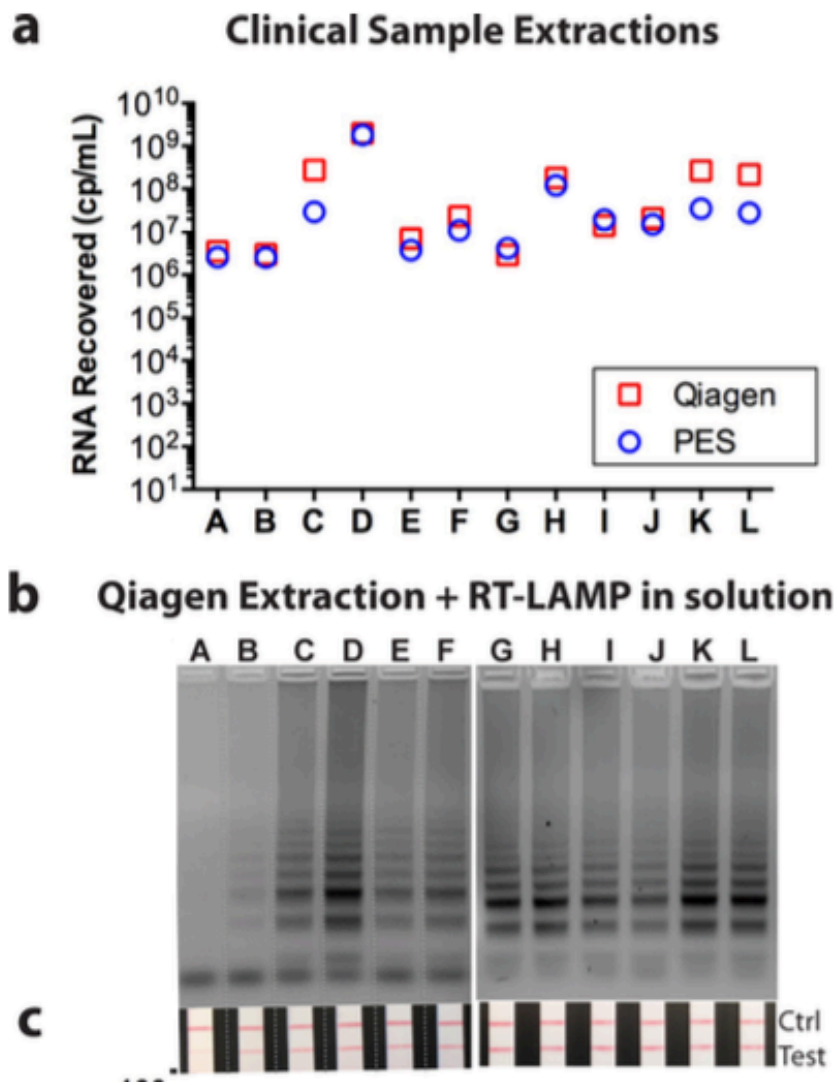
Linnes, et al., RSC Adv. 2014 Jan 1;4(80):42245-42251.

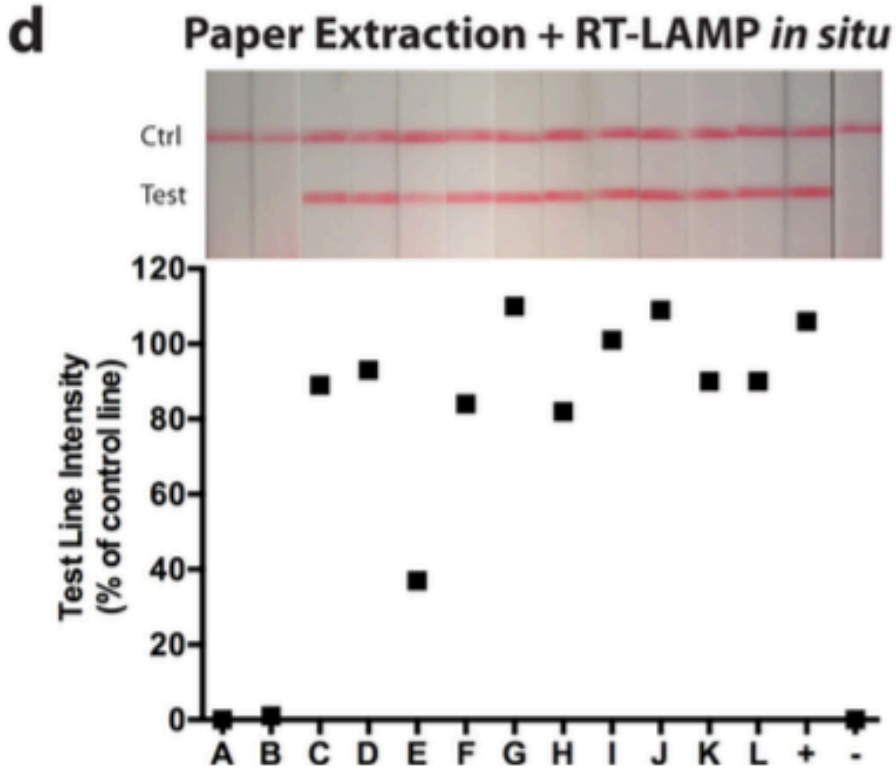
Readout


Clinical sample with analyte
mAbs to analyte - colloidal gold
Test line - mAbs to analyte
Control line
Capillary flow
Sample pad
Wicking pad
Capillary flow
Test line (positive)
Control line (valid test)


Type III
Amplicon 3
DNP-Probe
3'
5'
Biotin-Primer
Amplicon 2
DIG-Probe
3'
5'
Biotin-Primer
Amplicon 1
FITC-Probe
3'
5'
Biotin-Primer
C
T2
T1
flow

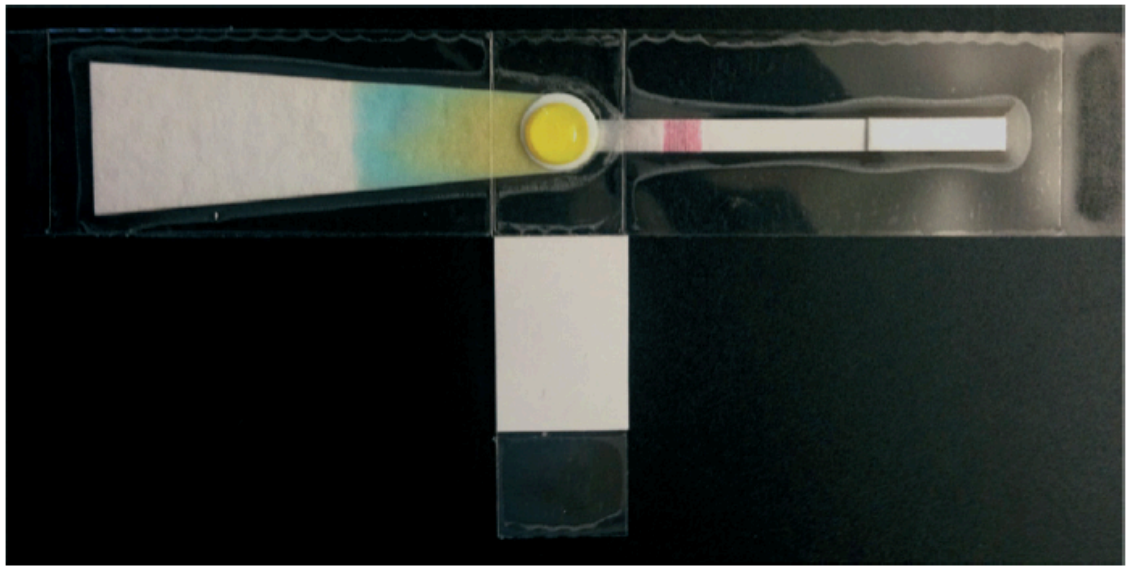
Clinical Influenza A Samples: Isothermal Amplification and Paper Readout





Rodriguez et al., Anal. Chem., **2015**, *87* (15), pp 7872–7879

Integration for HPV 16 Detection in Cervical Swab Samples

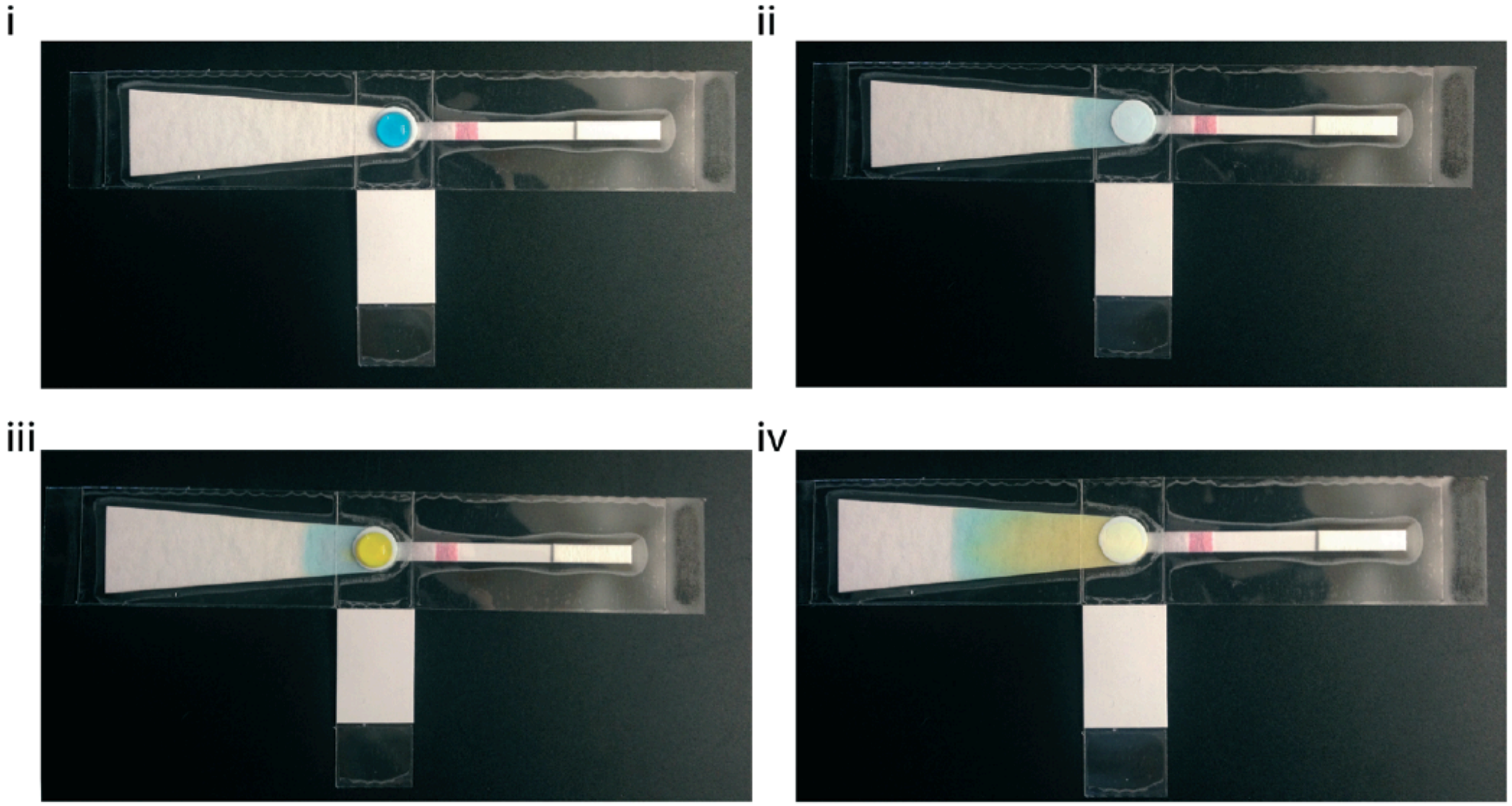


A fully integrated paperfluidic molecular diagnostic chip for the extraction, amplification, and detection of nucleic acids from clinical samples

Natalia M. Rodriguez, Winnie S. Wong, Lena Liu, Rajan Dewar and Catherine M. Klapperich*

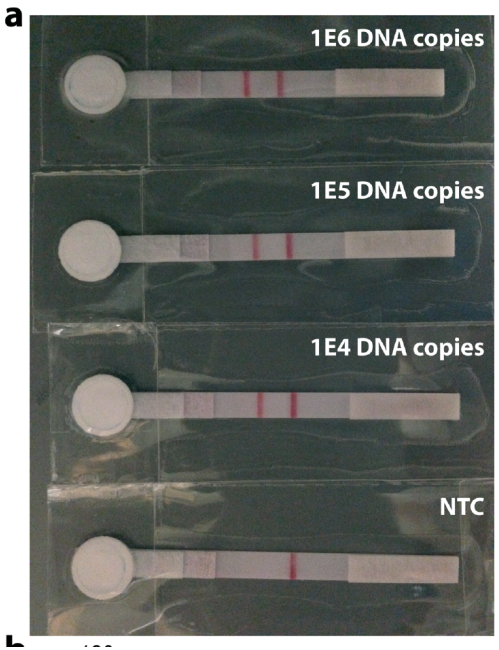
We present a low-cost, disposable, and fully-integrated paperfluidic molecular diagnostic chip for sample-to-result functionality at the point-of-care.

Running the Chip

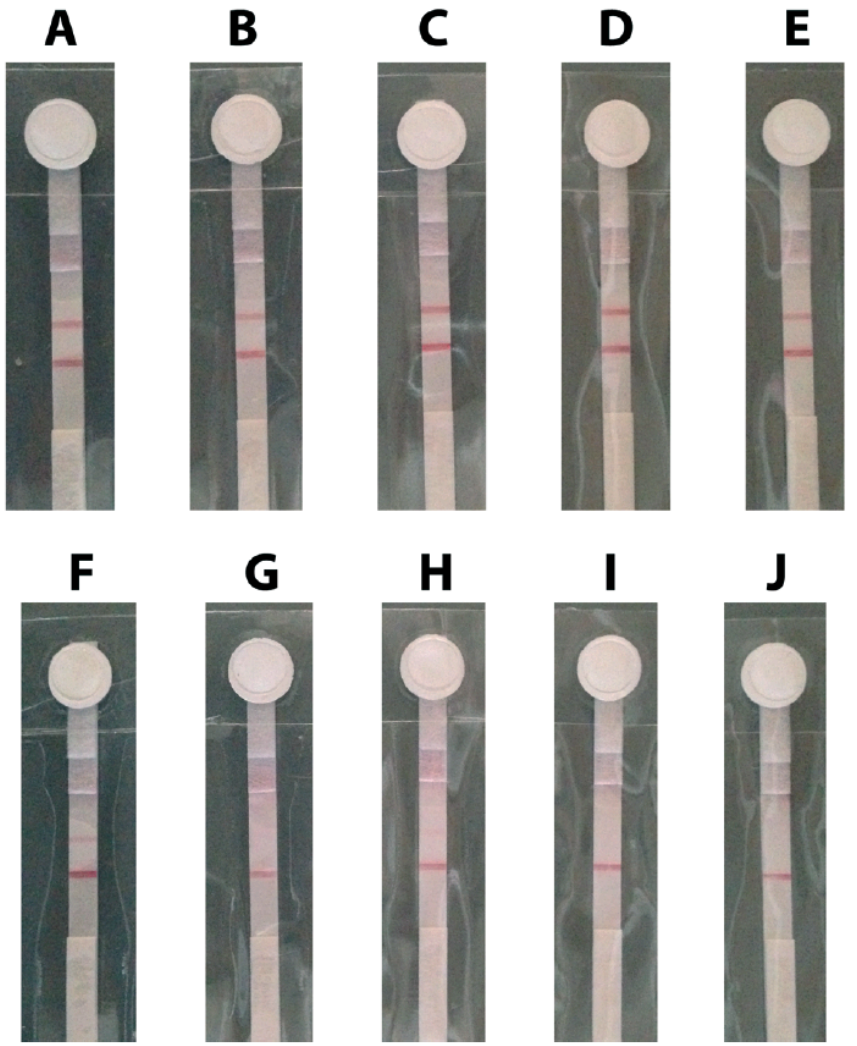


N. M. Rodriguez, et al. ***Lab Chip****, 2016,* ***16****, 753-763*

Results

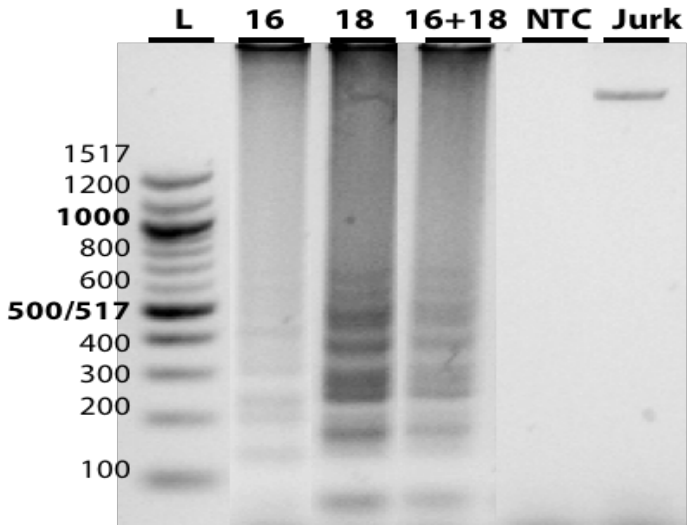


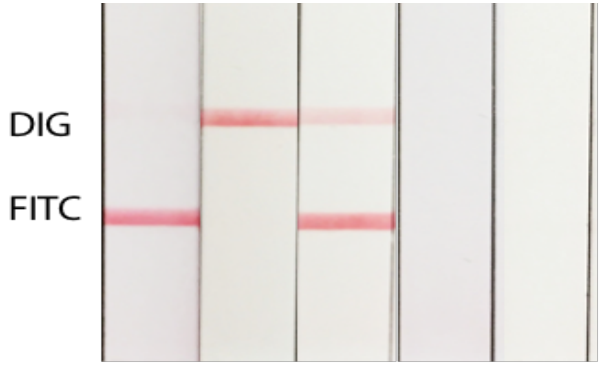
Sample ID	HPV 16 Quantity Mean (total DNA copies)	RNaseP Quantity Mean (total DNA copies)
A	9.00E+06	4.54E+04
B	1.89E+05	1.78E+04
C	6.04E+06	6.58E+05
D	5.08E+07	5.48E+05
E	1.27E+05	7.52E+04
F	NEG	1.39E+05
G	NEG	2.16E+05
H	NEG	1.70E+05
I	NEG	1.30E+05
J	NEG	1.07E+06



N. M. Rodriguez, et al. ***Lab Chip****, 2016,****16****, 753-763*

Multiplexing HPV 16 and HPV 18





FITC/DIG strips

JaneDx, Inc.

Incubating at the BU Photonics Center

Rajan Dewar, M.D.

Catherine Klapperich, Ph.D.

Mario Cabodi, Ph.D.

Natalia Rodriguez, Ph.D.

Customer & Value Chain

- <u>Target Customers and Value Chain Dynamics</u>:
  - ~~US path labs/Consumer play~~
  - General strategy for LMIC:
    - Ministry of health
    - NGOs
    - Providers
    - Patients

- *<u>First target market</u>:* ***India***
  - ***Private practices and distributors will buy from us and sell directly to patients.***
  - *We will thus target* ***distributors***, ***private practice*** *providers, then* ***state government***
  - *distributors (price, performance) → users (price)*

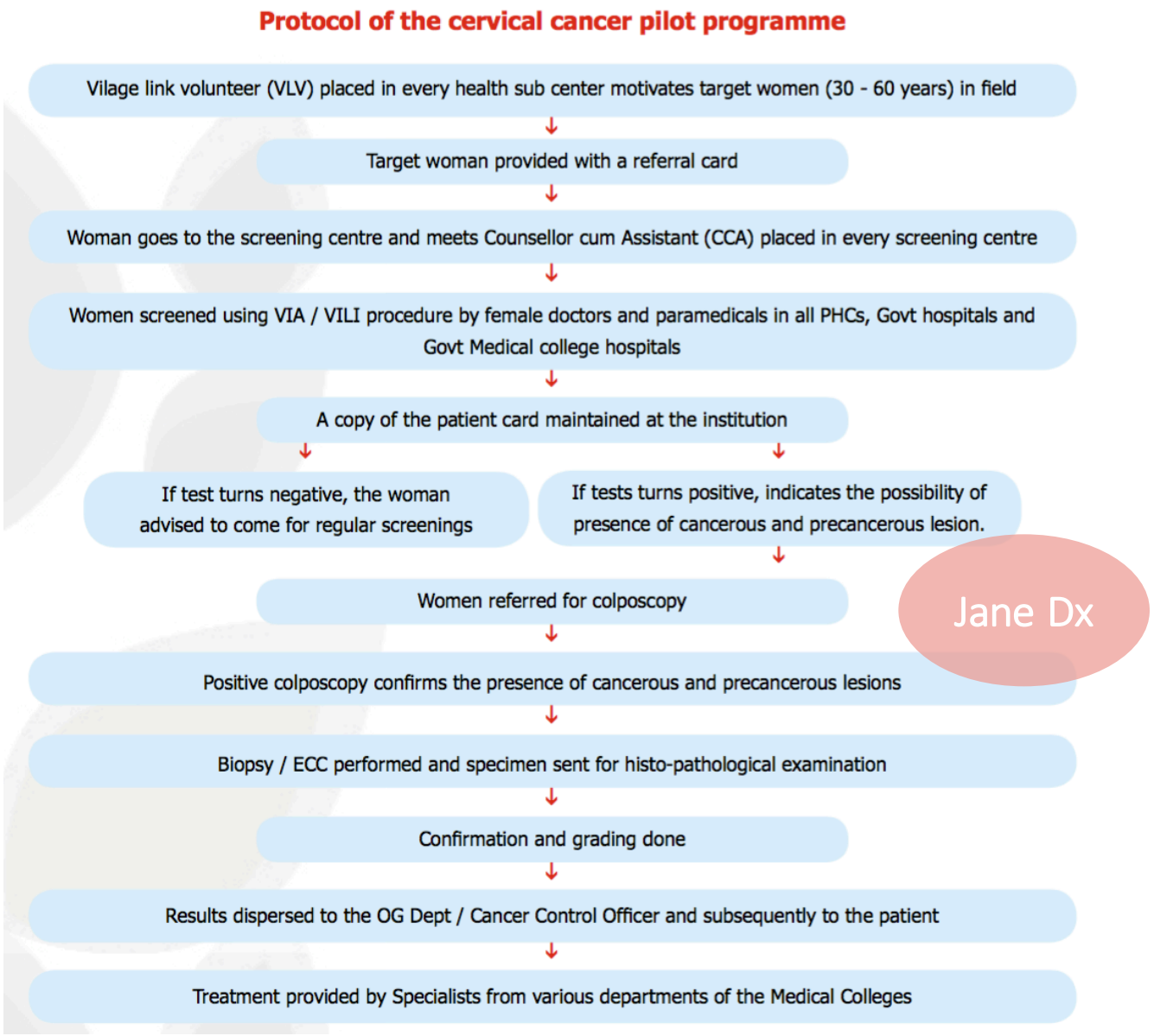
Revenue Model & Potential Market Size:

- Reimbursement: LMIC markets are highly segmented:
  - Insurance / reimbursement structure nearly non-existent (country-dependent)
- **Will patients pay Out-of-pocket for tests?**
  - India: growing middle class pays 100% oop for tests already
  - ***Very sensitive to price***
- Revenue Potential: ***India***
  - Customers would pay $10 distributors per test (assuming 100% markup); distributors buy from JaneDX at $5 per test.
  - Screening guideline: Women ages 35 – 64, cytology every 3 years; ***344 million women are eligible for screening***
  - Test 110 million women every year → $550M/year potential revenue

Feasibility Study Late 2016

1000 patients, 3 month Pilot. Integrated into Current Screening Program.

Tamil Nadu – Pilot Program



Jane Dx

More women die from cervical cancer every year in India than anywhere else in the world, according to the Cervical Cancer-Free Coalition

Total number of target women screened:
2,91,525 + 1,96,559
$1.5 per patient

POCTRN Resources Leveraged

- CFTCC Alpha Prototyping Core to Make Early Chips for Proof of Concept.
- CIMIT Primary Care Center Pilot Grant
- CIMIT CRAASH Course and Mentorship
- CFTCC Clinical Needs Assessment Core for India Market Research/Contact Establishment.
- JHU STI Center Discussions/Mentorship/Training Re: Sample Preparation of Cervical Swabs.

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Prof. Mario Cabodi

Alumni
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Dr. Jason Keller
Dr. Cassandra Noack
Samantha Byrnes
Dr. Jaephil Do
Michelle Wong
Dr. Mincheol Kim
Dr. Shichu Huang
Nga Ho
Dr. Qingqing Cao
Dr. Sonali Mahalanabis
Dr. Jane Zhang
Dr. Arpita Bhattacharyya
Jared Saffie
Jake Trueb
Melike Karakaya

NIH National Institute of Biomedical Imaging and Bioengineering
Creating Biomedical Technologies to Improve Health

POCTRN

CFTCC CENTER FOR FUTURE TECHNOLOGIES IN CANCER CARE

NIH U54 EB015403
NIH R01 EB008268
NIH R44 AI073221
NIH R21 AI071261
NIH R41 AI092913

www.bu.edu/cftcc
www.bu.edu/klapperich