Isothermal HPV 16 mRNA Amplification for Cervical Cancer Screening in Resource-Limited Settings

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Disclosures

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Overview

- Background: HPV DNA test
- **Point-of-care E7 mRNA test**
  - Goal: detection of HPV 16, 18 mRNA for more specific cervical cancer screening
  - Proof-of-concept testing on HPV 16 E7 gene alone
  - Isothermal amplification; lateral flow detection
  - Laboratory-based in current form

Background: Commercially available HPV DNA tests

Digene HC2

Qiagen, 2017

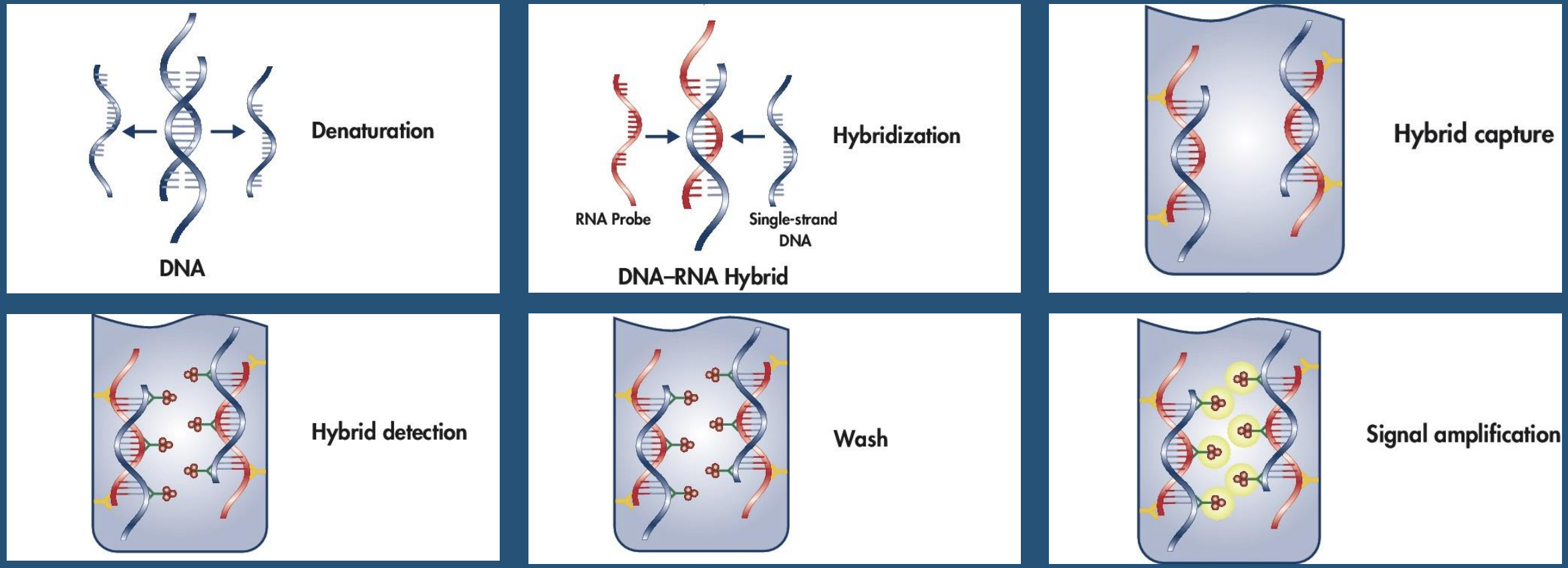
careHPV

Qiagen, 2017

Gene Xpert

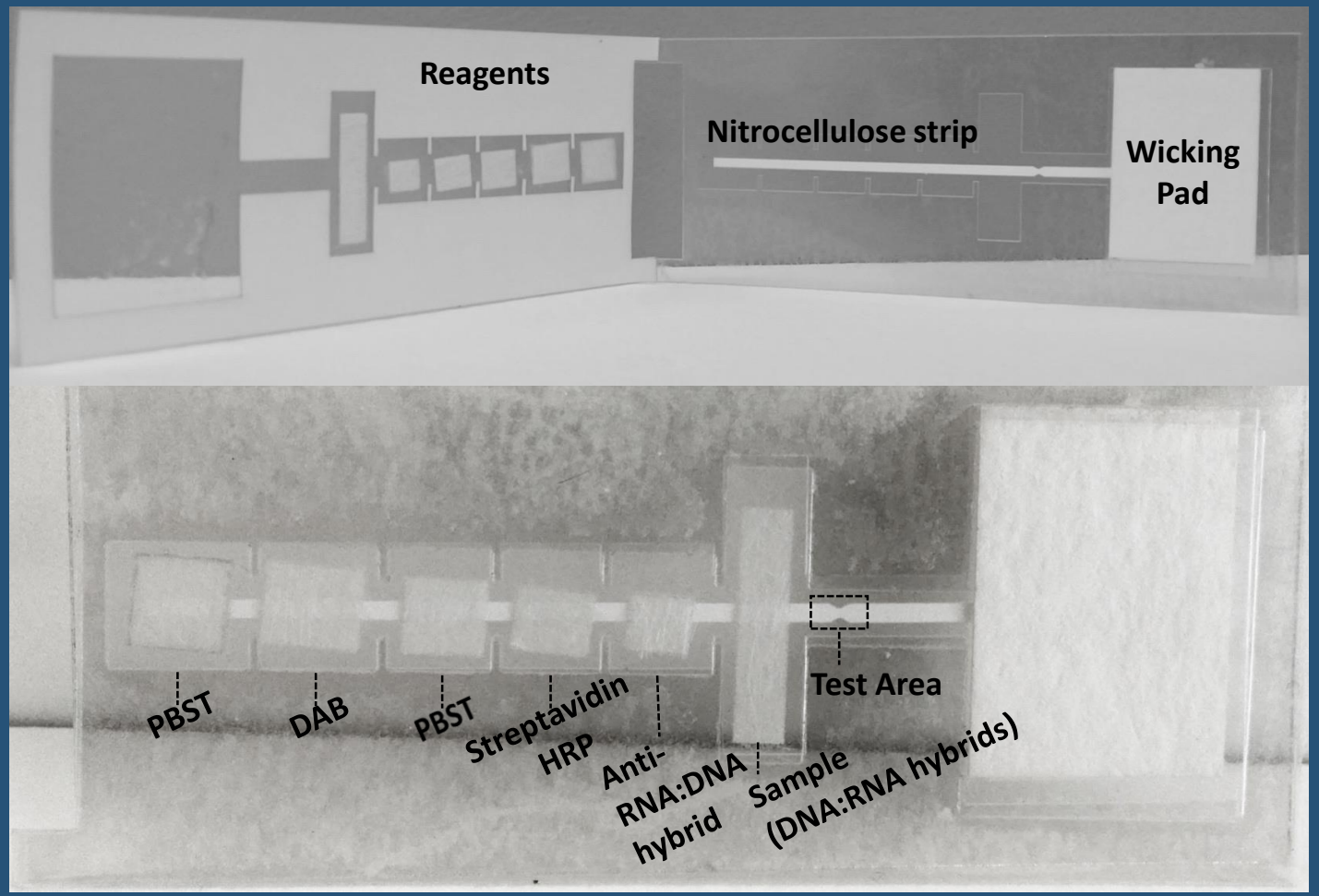
Cepheid, 2018

Background: Hybrid capture approach



Qiagen, 2018

Background: Paper-based HPV DNA test

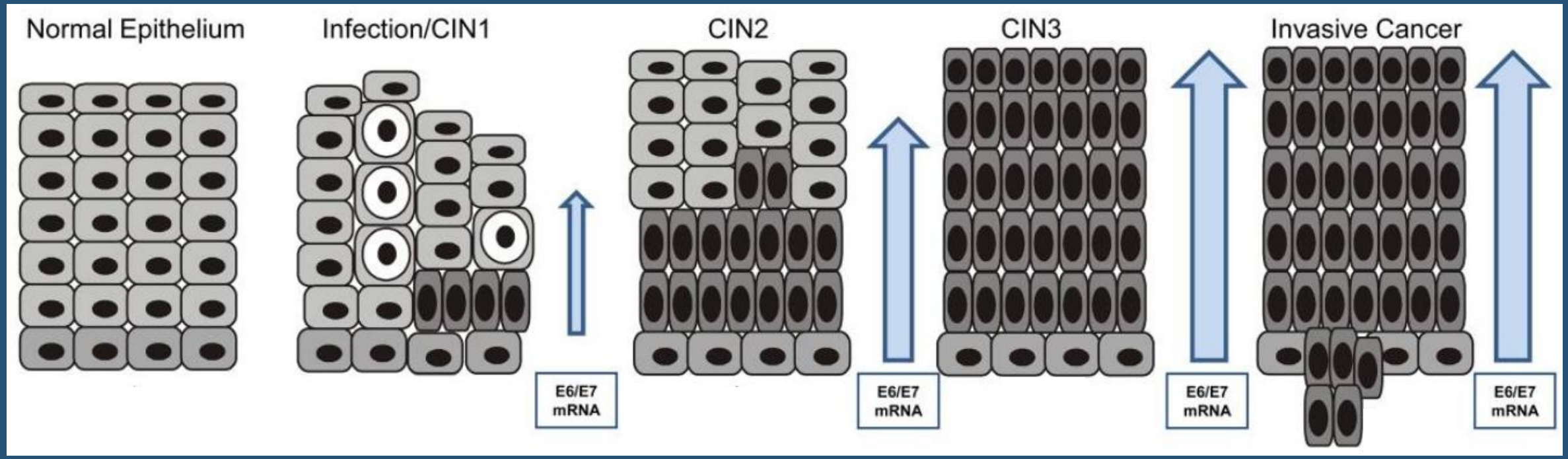


Background: HPV DNA test comparison

	Digene hybrid capture 2 (HC2)	careHPV	*Our Test*
Time (hrs)	4.5+ hours	3+ hours	***1 hour***
Cost/Test	$40-60	$5 (batch of 91)	***$2-3***
Need for Equipment?	orbital shaker, plate reader, heater	orbital shaker, plate reader, heater	***heater***
Need for Trained Personnel?	yes	no	***no***

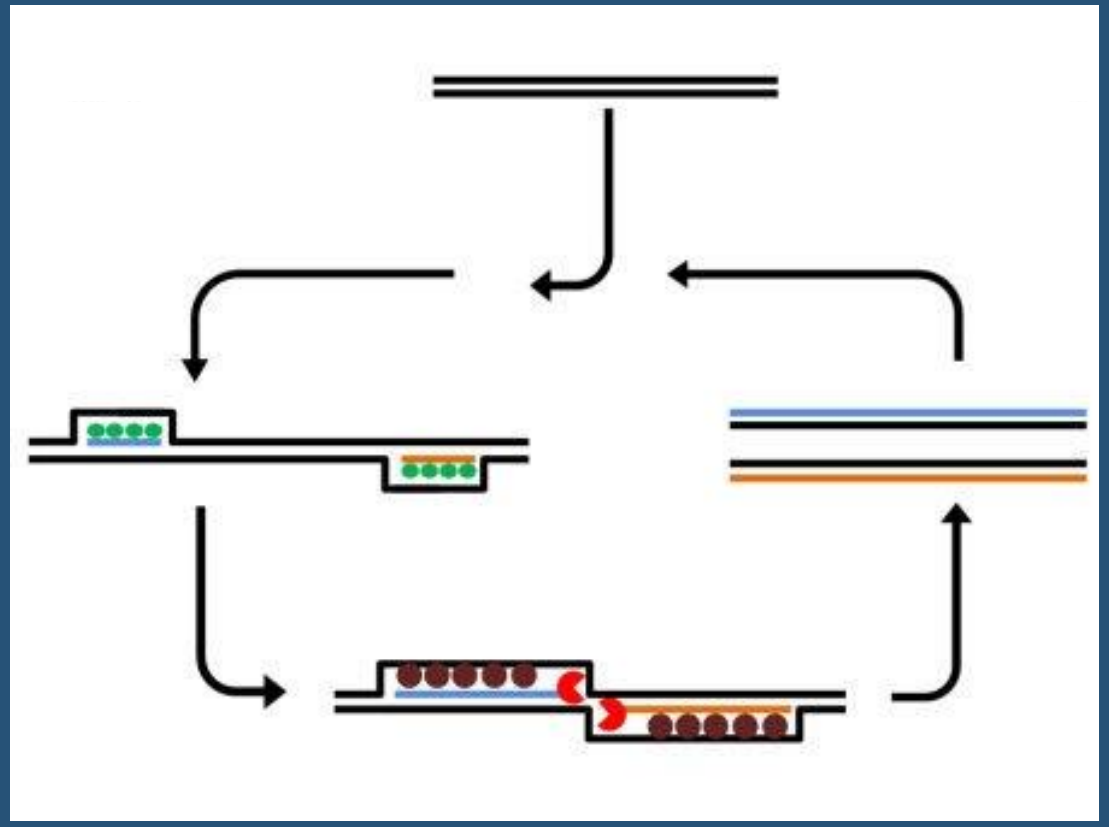
Point-of-care E7 mRNA test

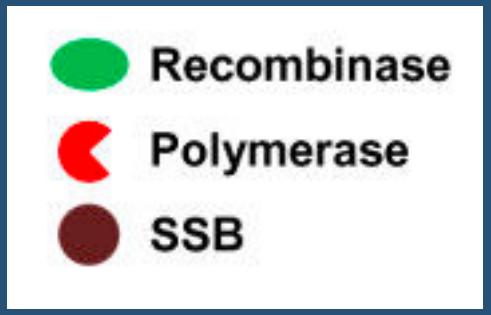
Potential for cervical pre-cancer molecular screening with high sensitivity and specificity



Adapted from Cuschieri and Wentzensen, *Cancer Epidemiol Biomarkers Prev,* 2008

Recombinase Polymerase Amplification (RPA)

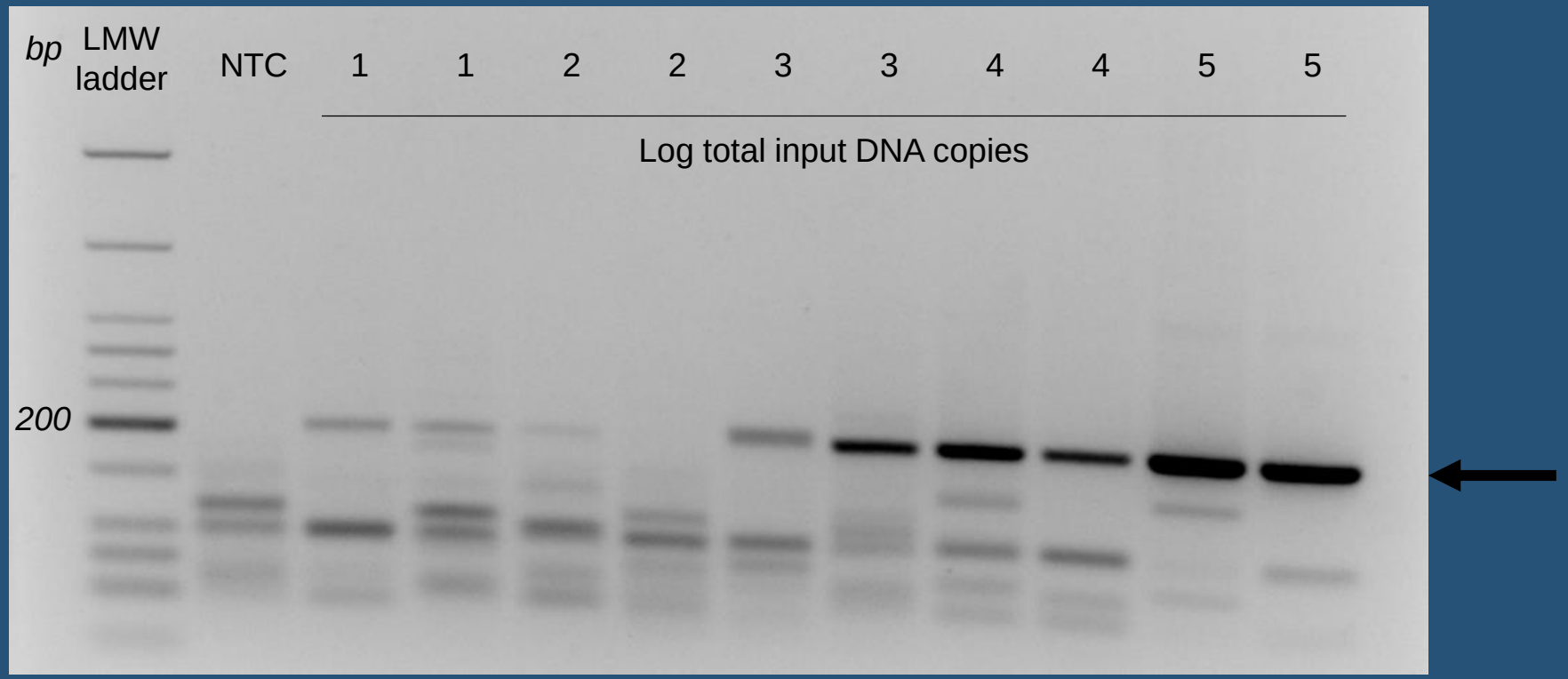




37°C
30 minutes

Adapted from Kersting et al, *Malaria Journal,* 2014

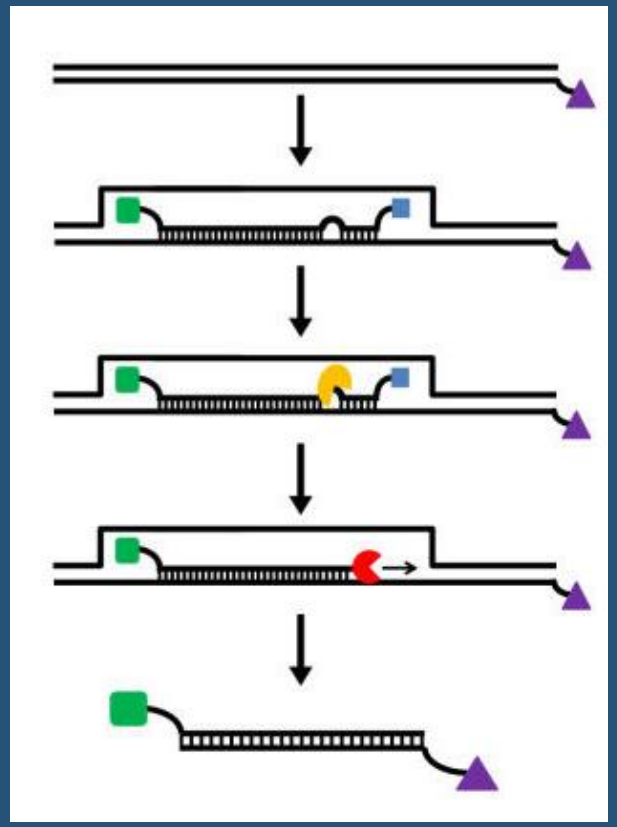
Isothermal HPV 16 DNA amplification (RPA)



Two-step HPV 16 mRNA reverse transcription and isothermal amplification (RPA)

cDNA from SiHa cells
~50 total copies expected

RPA nfo: lateral flow amplicon detection

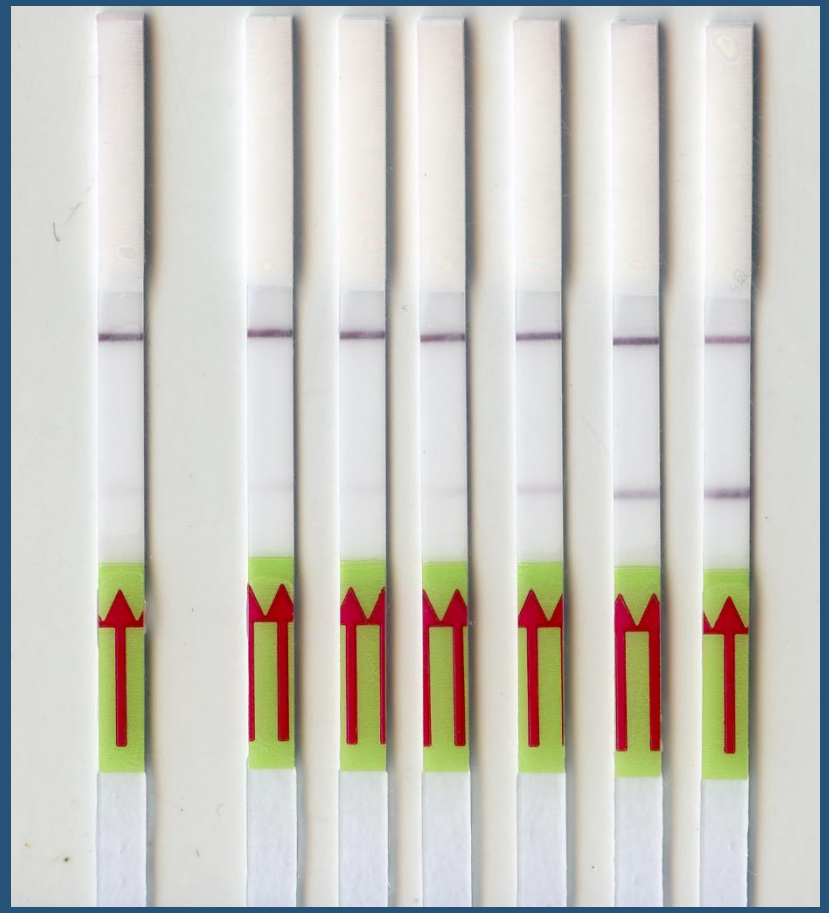


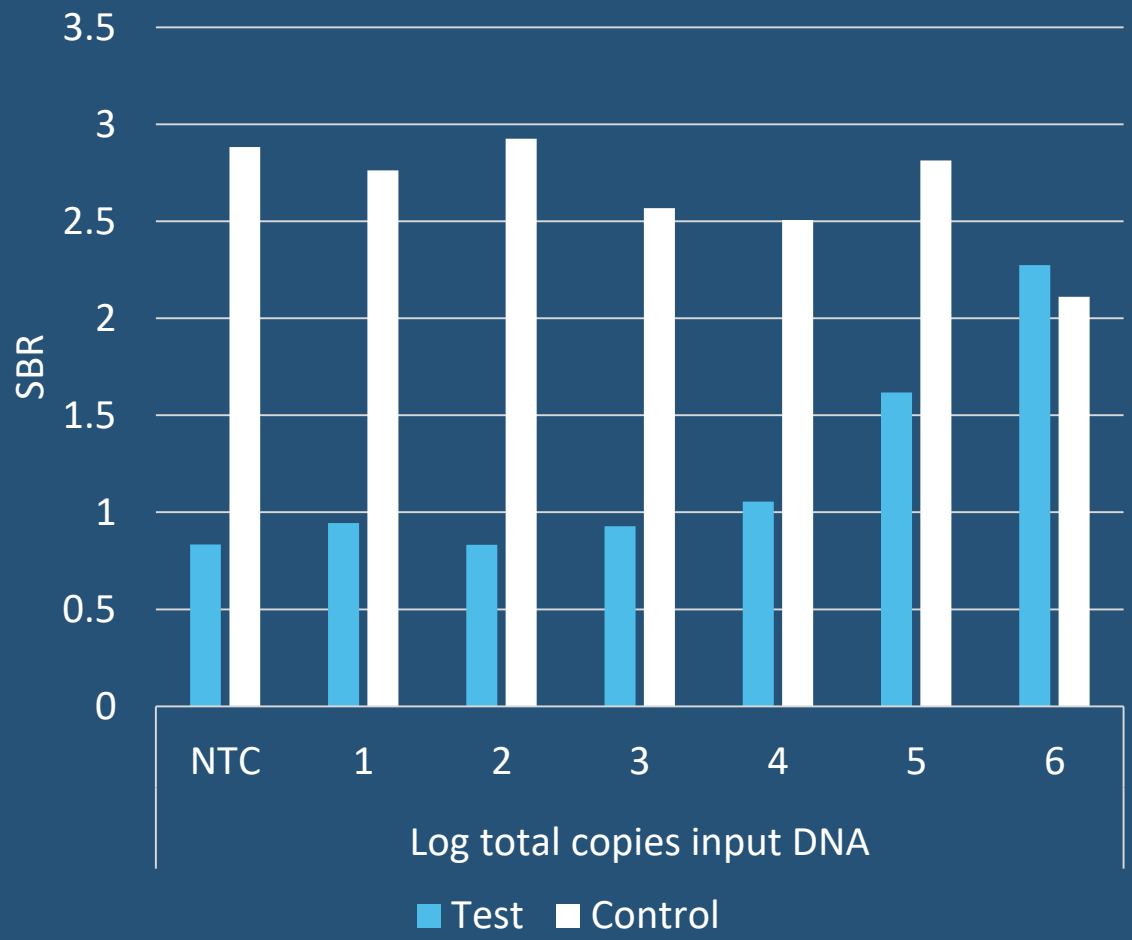
Kersting et al, Malaria, 2014

Agarose gels require 1-5 ng of DNA

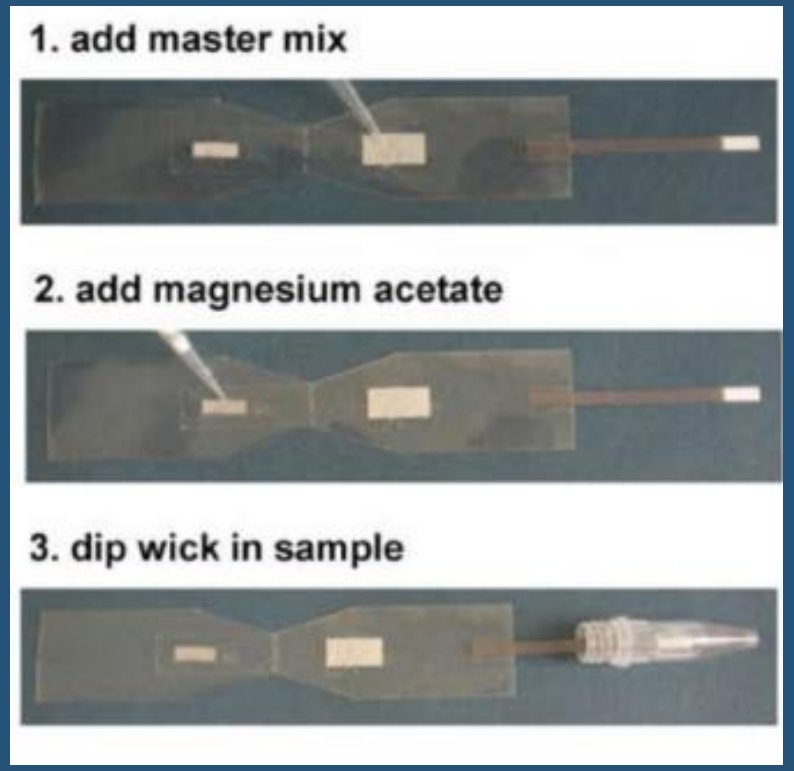
Lateral flow strips require 5 pg of DNA

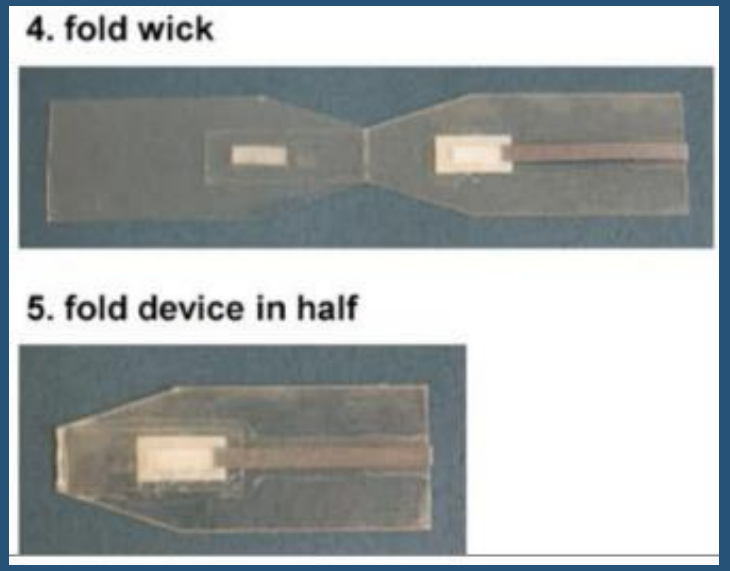
Paper-based HPV 16 E7 amplicon detection





Previous work: paper and plastic RPA device





B Rohrman and R Richards-Kortum, *Lab Chip*, 2012

Future work

- Improve paper-based amplicon detection sensitivity; reduce nonspecific product formation
- Multiplex HPV 18 amplification reaction
- Design continuous reverse transcription, amplification, and detection device
- Address sample preparation challenges
- Test clinical samples

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