SUPPLEMENTARY MATERIALS

HPV16 double stranded DNA	5'- CCC GAA AAG CAA AGT CAT ATA CCT CAC GTC GCA GTA -3'
HPV16 RNA	5'- rUrArC rUrGrC rGrArC rGrUrG rArGrG rUrArU rArUrG rArCrU rUrUrGrCrUrU rUrUrC rGrGrG -3'
HPV6 double stranded DNA	5'- ATC AAA GTG TCT ATA TTG GTT AAT TTT TCC ATG AAA -3'

Table S1. Short synthetic HPV16, RNA 16, and HPV6 DNA targets.

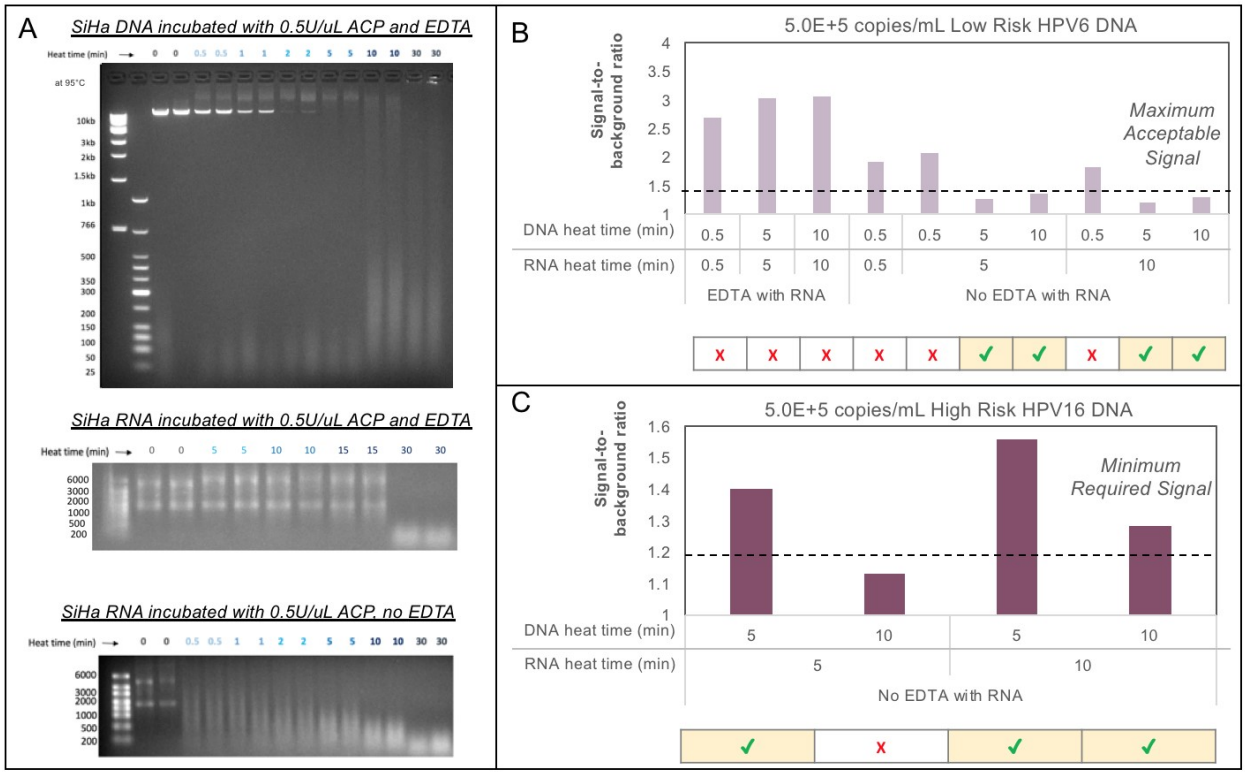


Fig. S1: Effect of DNA/RNA fragment size on paper HPV DNA assay performance**.** (**A**) Effect of heating time and EDTA on DNA and RNA fragment size. (*Top*) SiHa DNA incubated with 0.5 U/uL ACP in 1 mM EDTA; (*middle)* RNA incubated with 0.5 U/uL ACP in 1 mM EDTA*;* and *(bottom)* RNA with no EDTA incubated with 0.5 U/uL ACP for 5 minutes at 23 ºC, heated at 95 ºC for various times, and run on agarose gels. (**B**) Result of paper HPV DNA assay for a negative control (5.0E+5 copies/mL of low-risk full length HPV6 DNA) hybridized with full genome high-risk RNA templates. Prior to hybridization, DNA and RNA templates were incubated with 0.5 U/uL ACP for 5 minutes at 23 ºC, heated at 95 ºC for various times (n=2). Conditions resulting in unacceptable false-positive results are indicated with red Xs; conditions yielding an acceptable negative control signal are shown with green check marks. (**C**) Result of paper HPV DNA assay for a positive control (5.0E+5 copies/mL of high-risk full-length HPV16 DNA) hybridized with full genome high-risk RNA templates performed for acceptable conditions identified in (**B**), (n=2). Signal was strongest for high-risk HPV16 DNA with a 5-minute DNA and 10-minute RNA heat time. These heating conditions describe the RNA pre-fragmentation preparation practice prior to lyophilization as well as point-of-care sample preparation.

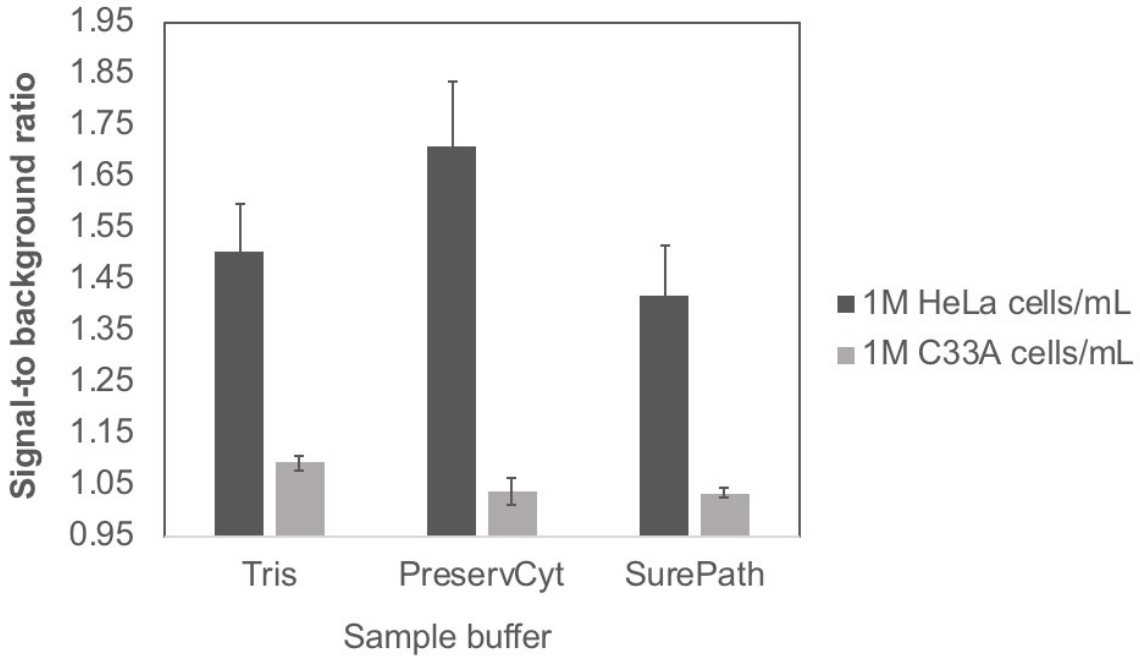


Fig S2: Assessment of paper HPV DNA assay with HPV-positive and HPV-negative cellular samples prepared in different collection buffers**.** Signal-to-background ratio for the paper HPV DNA assay was evaluated with HeLa (HPV18) and C33A (HPV-negative) cells prepared in a Tris-based buffer and in two sample collection buffers commonly used with clinical HPV DNA tests. Cellular samples were stored for >48 hours in either PreservCyt or SurePath buffer, converted to a Tris-based solution, and then tested on the paper HPV DNA assay. Following conversion to a Tris-based solution, comparable results were obtained using the paper HPV DNA for both sample collection buffers.

Site	Urban or Rural	Occupation
El Salvador (n= 30)	Urban (n=10)	Physicians (n=8)
		Nurses (n=1)
		Lab Technician (n=1)
	Rural (n= 20)	Physicians (n=20)
Mozambique (n=14)	Urban (n=14)	Physician or Nurse (n=13)
		Lab Technician (n=1)

Table S2: Usability participants. Number of participants from each site, stratified by location in an urban or rural setting and by occupation.

Sample collection and preparation	ACP	$ 0.02
	Cervical brush	$ 0.19
	Tubes and pipettes	$ 0.23
Paper HPV DNA assay	RNA (estimated)	$ 0.01
	Antibodies	$ 1.85
	Paper and plastic	$ 0.34
	Other reagents	$ 0.33
Sample collection and preparation total		$ 0.45
Paper HPV DNA assay total		$ 2.52
Overall cost		**$ 2.96**

Table S3: Cost estimation using small-scale manufacturing.

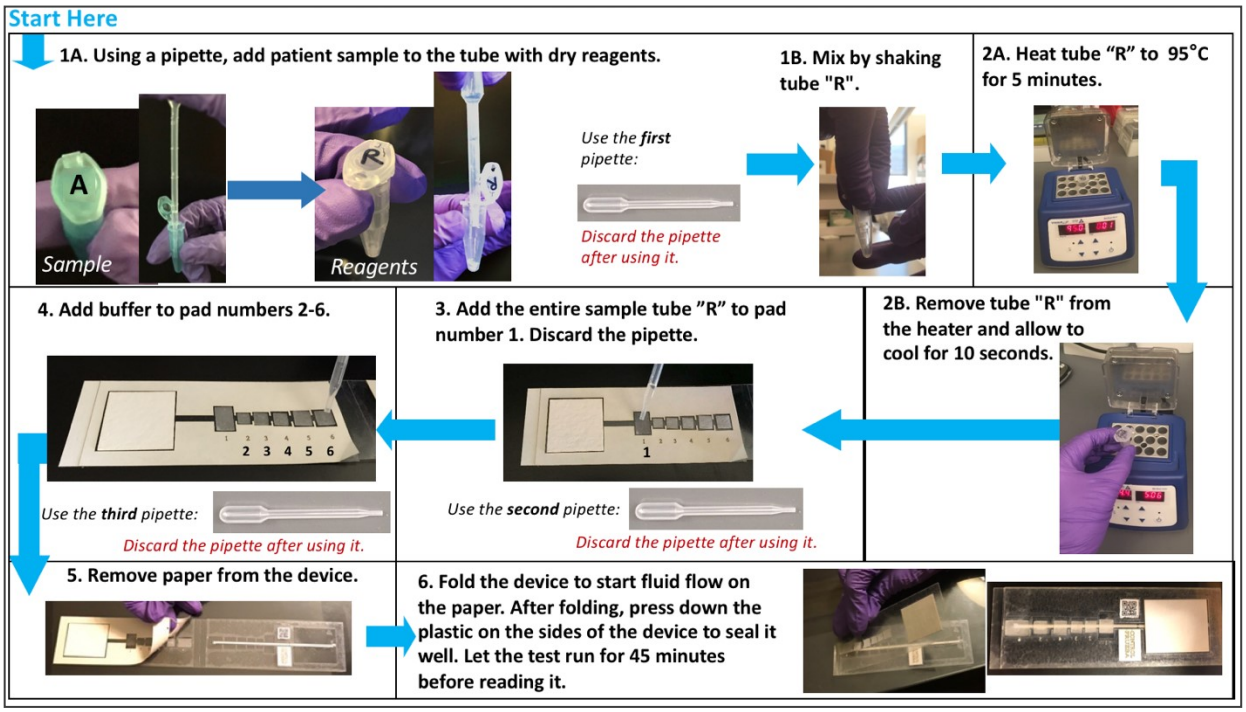
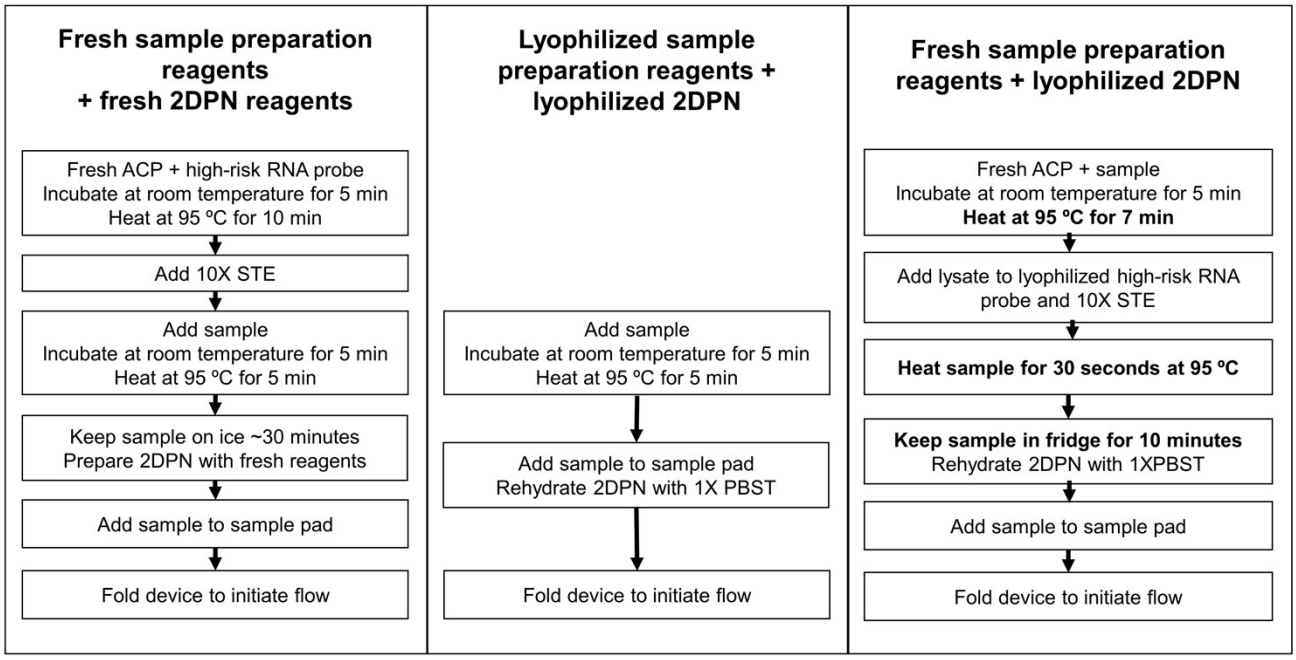


Fig S3: Job aid used during usability studies. Job aid was translated into Spanish for El Salvador studies and Portuguese for Mozambican studies.



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38 **Fig S4**. Comparison of sample preparation workflows during field validation in Mozambique. The
39 paper HPV DNA test with lyophilized sample preparation reagents and lyophilized two-
40 dimensional paper network (middle) were used to assess cellular controls during field testing in
41 Mozambique to generate results in Figure 7. Sample preparation was re-optimized during clinical
42 sample assessment to minimize false positives, hypothesized to be due to cellular RNA secondary
43 structure. The re-optimized workflow (right) was used to generate results in Figure 8, with changes
44 highlighted in bold, compared to the sample preparation protocol used during controlled laboratory
45 testing to generate results in Figure 6 (left).

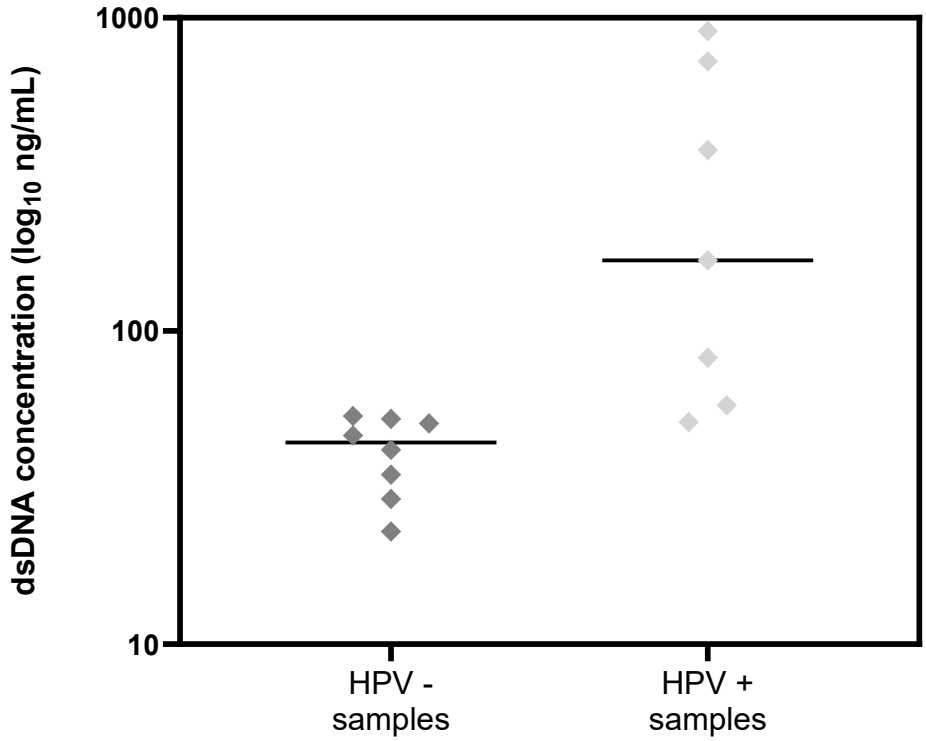

Fig S5. Double-stranded DNA (dsDNA) content of clinical samples with careHPV reference standard that were assessed by the paper HPV DNA assay in a controlled laboratory environment. The mean DNA content of samples that tested negative by careHPV was significantly lower than that of samples that tested positive by careHPV (p=0.031, two-tailed unpaired t-test).

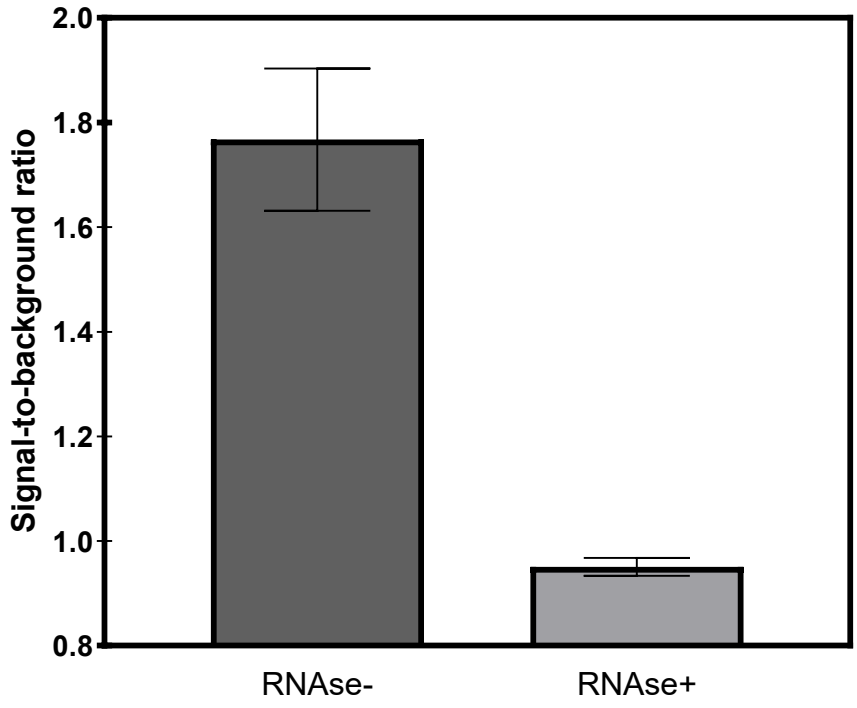

Fig S6. Effect of RNAse treatment on paper HPV DNA assay signal formation. Results of the paper HPV DNA assay performed with C33A (HPV-negative) cells at 10^7 cells/mL, with and without RNAse A treatment during sample preparation (n=2 per condition). Cells were incubated with either 0.5 U/uL ACP (RNAse-) or 0.5 U/uL ACP and 6uL of RNAse A (RNAse+) for 5 minutes at 23 ºC, followed by heating at 95 ºC for 5 minutes. High-risk RNA probe was **not** hybridized to C33A sample before addition to the sample pad.