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Supplementary methods

Replicate quantification experiments

A series of experiments was completed to determine the reproducibility of quantification by the qPCR and LAMP assays. All experiments were conducted on human biopsy samples. For technical replicates, we amplified each human sample twice on the same qPCR plate, and quantified the samples by comparison to standard samples amplified on the same plate. For experimental replicates, a plate containing all human samples and all standard samples was amplified in two separate experiments, using the same qPCR master mix. Each plate was then quantified using an average standard curve. An average standard curve was used to mimic the quantification method we used in TINY, as TINY's limited throughput (4 samples/test) does not enable standard samples to be amplified in the same experiment as human samples. Master mix was frozen and thawed between experimental replicates for the qPCR assay. For the LAMP assay, technical replicates were performed in the ViiA 7 (two of each human sample was amplified in the same experiment), and experimental replicates were performed in TINY (human samples were amplified in different experiments, but using the same master mix, which was frozen and thawed between replicates).

Photodiodes used in TINY

The photodiodes in use (TSL237SM, ASM sensors) are capable of transducing small light signals to a square wave signal with frequency proportional to irradiation. We used light-to-frequency converters over light-to-voltage converters so that the resolution of the measurement would not be limited by the analog to digital converter of the microcontroller (Teensy 3.2). A Teensy was used because it is capable of simultaneously measuring frequencies from multiple inputs, with a standardized frequency measurement library.

Threshold time calculation from photodiode data

We first smoothed each frequency using a 10-point moving average method. Then, the Evagreen fluorescence (blue LED) and absorbance (red LED) smoothed frequencies were normalized by the ROX smoothed frequencies (yellow LED). Two different algorithms were used to calculate threshold time from this normalized frequency data. In the first method, a line was fit to the pre-exponential-amplification normalized frequencies, and the difference between the normalized frequency and the fit

was calculated (Fig. S4). Once exponential amplification began, this difference would raise above a threshold and the algorithm would calculate this time as the threshold time. The second algorithm calculated the difference in normalized frequency between successive data points (Fig. S5). Since the second algorithm did not depend on a fit line, we found it to be more reliable. All threshold times reported from TINY were calculated using this second method.

Phase change material selection

During initial prototyping of TINY, we tested isothermal reactions with PureTemp 63 (nominal T_m: 63°C) and found that it was suitable for LAMP. However, we observed more instances of late-stage amplification of negative control samples when using PureTemp 63, and therefore chose to work with PureTemp 68 (nominal T_m: 68°C) for all experiments presented in this paper. Phase change materials were donated by Entropy Solutions LLC.

Solar absorber plate construction

The plate used for absorbing sunlight is an aluminum disk painted with flat, black paint. A Teflon o-ring and acrylic disk were fixed by high-temperature epoxy onto the top of the black aluminum disk. The acrylic disk functions to slow convective heat loss to the ambient. The Teflon o-ring has high temperature tolerance and serves to separate the acrylic disk from the hot absorber plate.

Solar heating of TINY

To heat TINY using sunlight, we first found a relatively flat working surface that had no obstructions of the sun. A support structure was attached to TINY that mounted a 28 x 28 cm square Fresnel lens (Edmund Optics part #32-597). Both the TINY and the support structure for the lens are capable of rotation, for manual alignment with the sun. After alignment, the lens was used to concentrate sunlight onto the absorber plate until enough heat was collected and the isothermal temperature was reached. Realignment with the sun was typically necessary between 1-3 times per experiment.

Hotplate heating of TINY

A micro hotplate from ThermoFisher (HP2305BQ) was used to heat TINY via electricity. To heat, a cutout in the bottom of the TINY enclosure was removed, and the bottom aluminum surface of the outer cylinder in TINY was set onto the hotplate. For the data displayed in Fig. 4A, both TINY and the

hotplate began at room temperature. The hotplate was then set to level 5 and TINY was placed on the hotplate for the reported heating time. After heating, the bottom aluminum cutout was reattached to slow heat loss to the ambient.

Bunsen burner heating of TINY

A portable, butane-fueled Bunsen burner (Fisher Scientific, item S65148) was used to heat TINY via flame. Three support legs were mounted to the bottom of TINY to raise the system an appropriate height above the Bunsen burner. Then, the cutout was removed from the bottom of the TINY enclosure to expose the bottom aluminum surface of the outer cylinder. The Bunsen burner was placed beneath this aluminum surface and turned on a low setting to heat TINY. After heating, the bottom aluminum cutout was reattached to slow heat loss to the environment.

Automated heating of TINY via cartridge heater

A 12-volt DC cartridge heater rated at 54 W (Comstat Inc., part MCH1-240W-004) was used for automated heating of TINY. An AC-to-DC adapter (12-volt) was used to power a central PCB with a switch, fuse, and MOSFET in series with the cartridge heater. The gate of the MOSFET was controlled by a digital signal from the Teensy microcontroller, which used a simple code to cycle the heater on or off based on the temperature of the outer cylinder and the temperature of the bottom PCB. We chose a surface-mount power MOSFET (IRLR7843PbF, International Rectifier) with low R_{DS} (2.6 mΩ) to try to minimize the voltage drop across the drain and source.

Lyophilized reagents used in Uganda

A two-part reaction was setup, and consisted of a lyophilization mixture and a rehydration mixture. The lyophilization mixture contained a final concentration of 1.4 mM dNTPs, 1.6 µM FIP/BIP primers, 0.2 µM F3/B3 primers, 0.4 µM LoopF/LoopB primers, 960 U/ml Glycerol-Free *Bst* 2.0 Warmstart DNA Polymerase (New England Biolabs, cat. no. M0402Z), 1X EvaGreen, and 1X ROX. This mixture was added in equal parts to 2X Lyophilization Reagent (OPS Diagnostics, cat. no. LR2X 500-02) before being frozen at -80˚C and then transferred for overnight lyophilization in a Labconco Freeze Dryer. After lyophilization, samples were vacuum packed and stored at room temperature. The rehydration mixture consisted of 1X Isothermal amplification buffer and 6 mM $MgSO_4$. Samples were stored at

room temperature for 8 days before experiments in Uganda, and were rehydrated immediately before performing the LAMP assay.

LAMP experiments performed in Uganda

We used the same operating procedures in Uganda as those outlined in the previous methods sections. For solar experiments, we heated TINY outside in the sun to a temperature slightly above 68°C. Following heating, TINY was brought back inside the clinic and then LAMP was performed with no further heat input. All LAMP reactions performed in Uganda were conducted for 50 minutes; however, samples which amplified past 24 minutes were considered negative. Since any amplification that started beyond 24 minutes was to be considered negative, we used 24 minutes as the time for TINY amplification during our timeline analysis for biopsy-to-result.

Field trial design

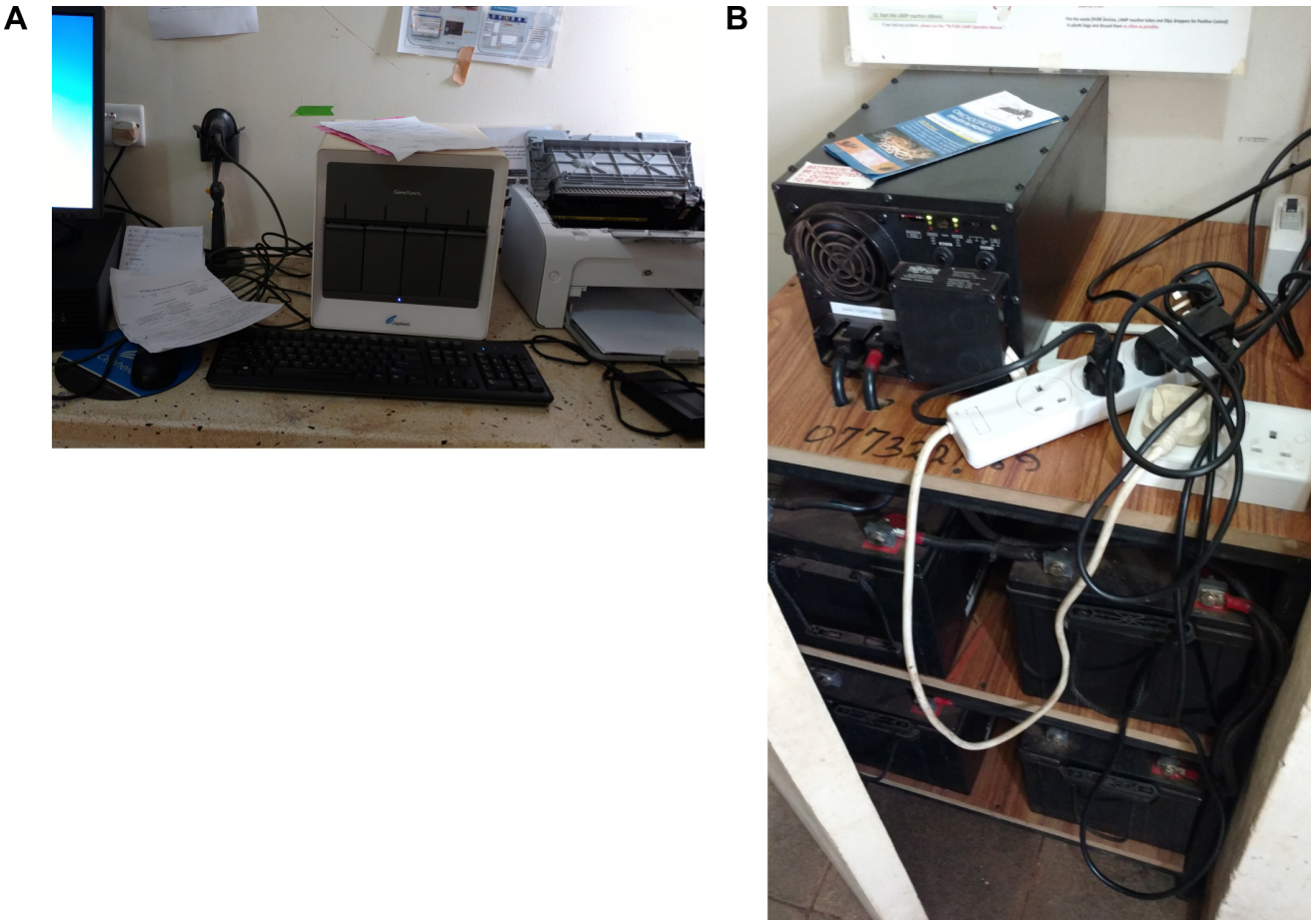
Our field trial took place over one week (five working days) in Uganda during November 2017. The field trial was planned to answer a series of hypothesis:

- Hypothesis #1: TINY will perform equivalently in Ugandan health clinics as in controlled conditions in the US.
- Hypothesis #2: TINY will perform equivalently using any heat source, including sunlight.
- Hypothesis #3: Local teams can be trained to use TINY and their results will match those obtained by Cornell staff.

Our plan was to analyze as many human samples as possible to test the above hypothesis, within our five-day trial. Since many of our desired experimental conditions could not be guaranteed (sunlight availability, electricity outages, patient presentation), we could not plan a rigorous schedule but instead performed experiments as the opportunity presented. When possible, we analyzed fresh biopsies from patients who presented during one of our working days in Uganda (three patients presented). We also analyzed five other samples which had been previously biopsied and stored in a freezer in RNAlater. We took five completely assembled TINYs to Uganda, leaving three to be used by the Ugandan team after our departure. After the field trial, biopsies continued to be taken from KS-suspect patients and stored for future analysis by the local team. Four months after the field trial, 21 patient samples were tested in TINY by the local team, who used the same operating procedure as outlined elsewhere in this

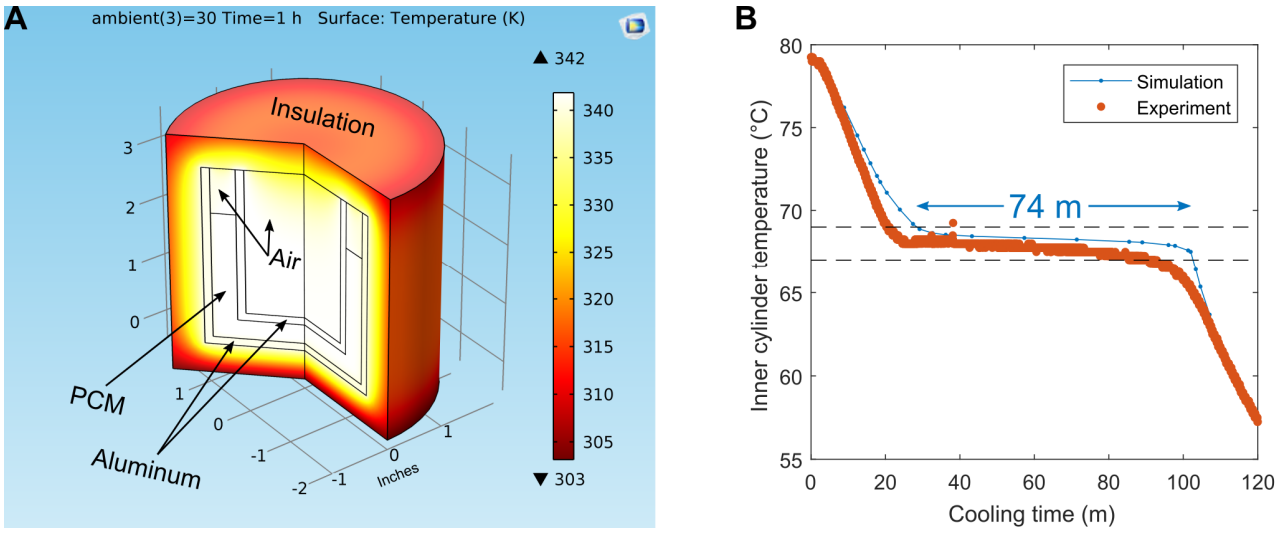
manuscript. TINY results were then sent digitally to the Cornell team, and DNA extractions performed in Uganda were sent to Weill Cornell for KSHV DNA testing by qPCR.

Supplementary figures

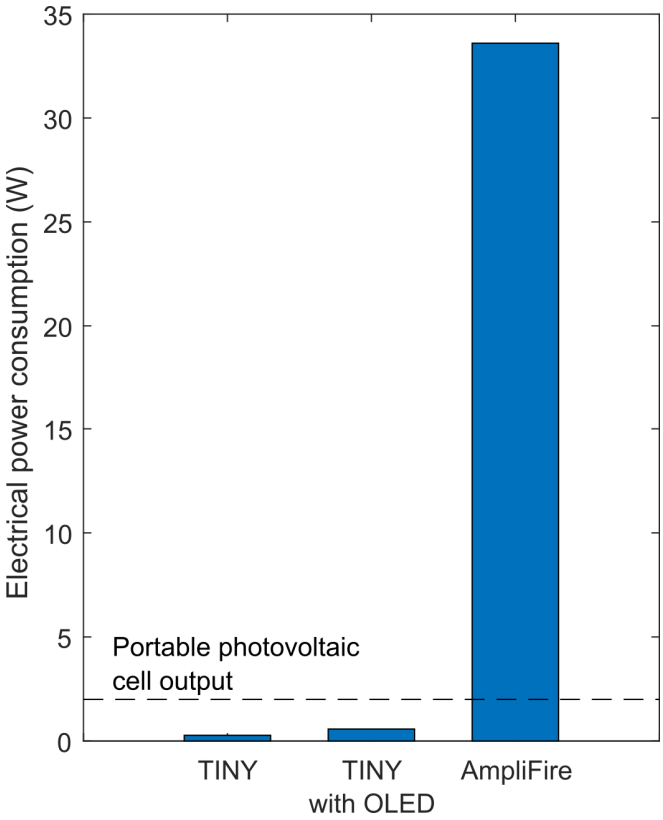


Supplementary Fig. 1. Battery backup for GeneXpert in rural Uganda.

In July 2016, our team traveled to Uganda to study the diagnostic equipment available to rural health clinics. (**A**) This GeneXpert IV was found at Kiboga Hospital, Uganda. A bank of batteries (**B**) was set up to power the GeneXpert during power outages, which were experienced weekly. GeneXpert operation was expensive relative to the hospital's budget, and so a battery bank was installed to prevent interrupted assays.

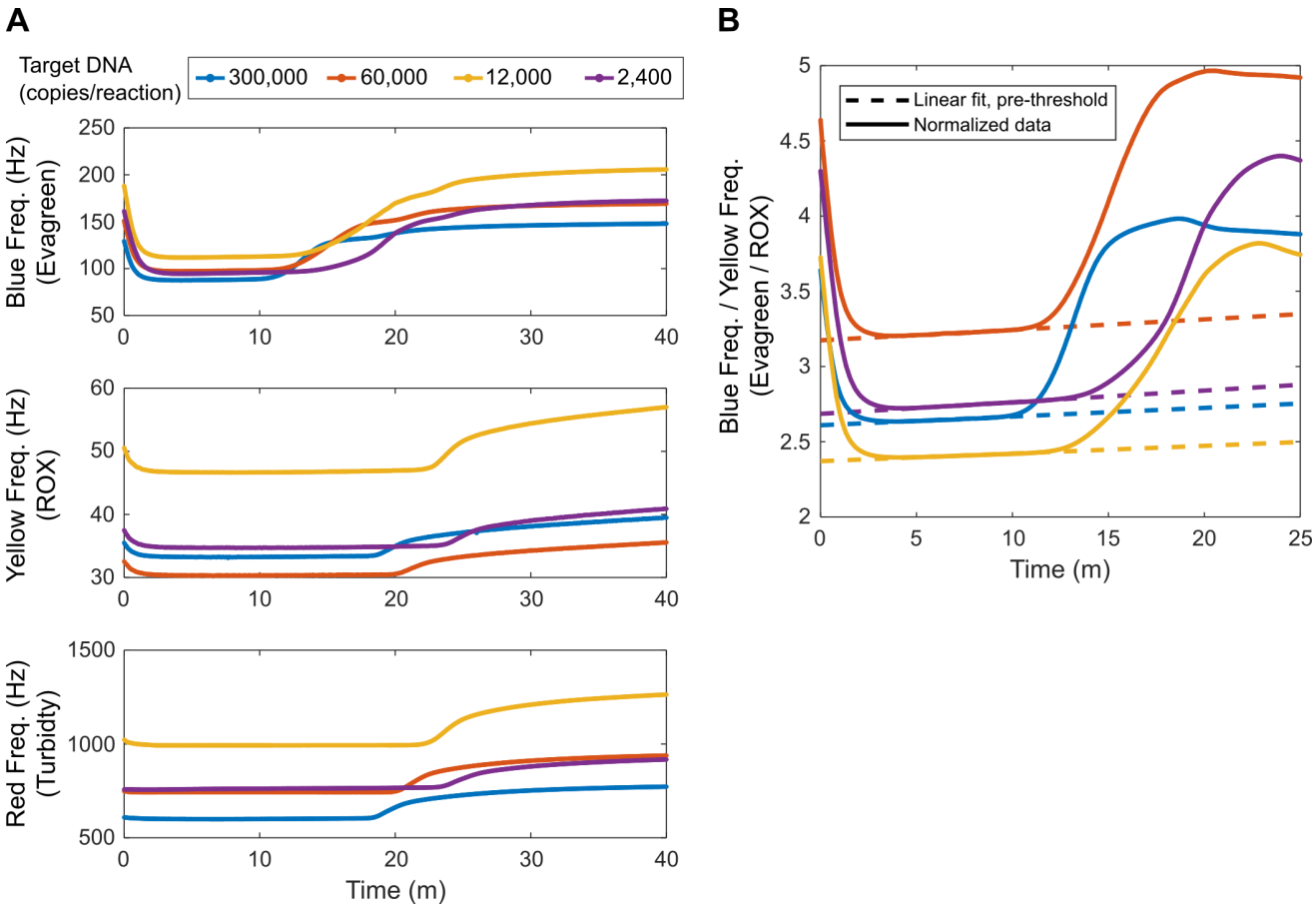


Supplementary Fig. 2. COMSOL simulation and experimental verification of isothermal dwell. (**A**) COMSOL was used to model the most critical heat transfer components within TINY. Boundary conditions: room-temperature on bottom, external natural convection on sides and top. (**B**) An early prototype of TINY was used to experimentally confirm the results of the simulation, showing that stabilization at 68 ± 1°C (dashed lines) for over an hour was possible. Experiment conducted once. This preliminary design of TINY was used to estimate critical design parameters (i.e. phase change material mass, insulation thickness), but does not match the final design exactly due to other constraints and features (e.g. the size of measurement unit, mechanical connections to the TINY enclosure).



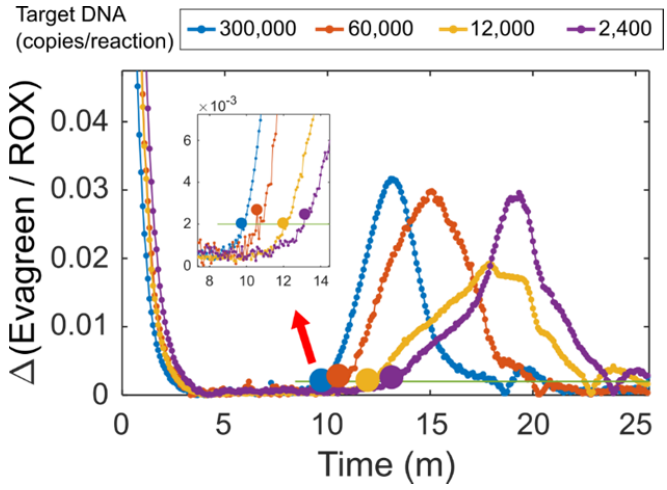
Supplementary Fig. 3. TINY's electronics can be powered by a small photovoltaic cell.

The power requirement of TINY with and without a display are well within the power output of a portable photovoltaic cell (e.g., 2 W, 110 mm by 140 mm, 90 g). TINY power consumption measurement error: ± 10 mW. We also analyze TINY with a display to consider the power requirement where all data processing and result communication could be achieved without a laptop (i.e. with TINY's microcontroller). The display power requirement was calculated with a SSD1306 OLED consuming 300 mW. While TINY can be heated via non-electrical sources (sunlight, flame), systems that must be heated via battery power (e.g. AmpliFire, Douglas Scientific) consume too much power to be operated in the field indefinitely by small photovoltaic cell, and instead can only operate between 4 to 6 hours per charge. AmpliFire specifications retrieved from the manufacturer's website.



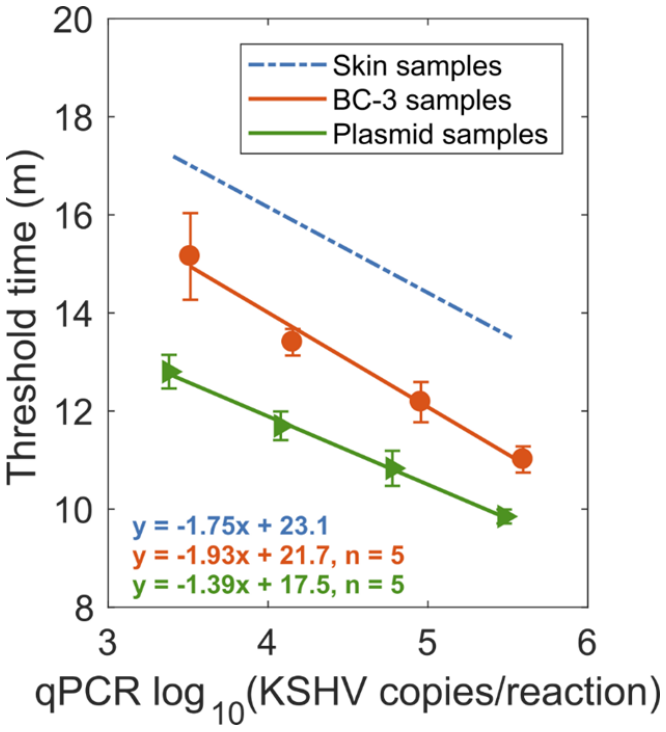
Supplementary Fig. 4. Raw photodiode data from TINY during the LAMP assay.

(**A**) Raw data is collected from photodiodes during three different LED excitation states (blue, yellow, red). Blue light excites Evagreen dye, yellow light excites ROX dye, and red light provides an absorbance measurement. Photodiodes convert irradiation to frequency, which we measured using the Teensy microcontroller. (**B**) When processing the raw photodiode data, we normalized the Evagreen values by the ROX values and then applied a fit (dashed line) to the linear portion of the response. The fluorescence data reported in Fig. 4c is the difference between the normalized data (solid line) and the fit line seen here. This data was collected from a TINY prototype with wells for four samples instead of six samples. Data from (A) and (B) are demonstrative, but a similar quality of measurement was maintained across all experiments in TINY.



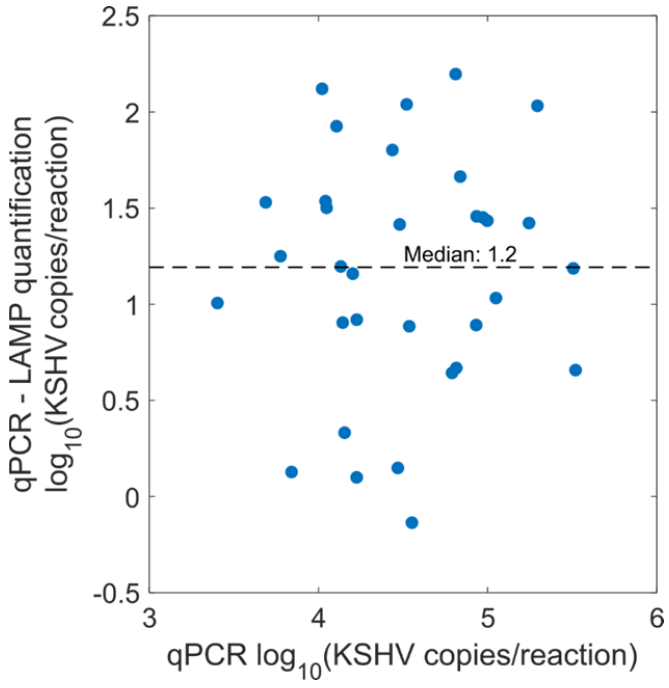
Supplementary Fig. 5. Calculation of threshold time via difference data.

The difference between successive points of the Evagreen data normalized by the ROX data. All TINY threshold times reported in this article were found via this method, and were calculated as the time that the data passed a pre-defined threshold (0.002: the green horizontal line). The large circles mark the calculated threshold times. We found this method of threshold time calculation the most reliable, as it did not depend on line fitting. This data was collected from a TINY prototype with wells for four samples instead of six samples.



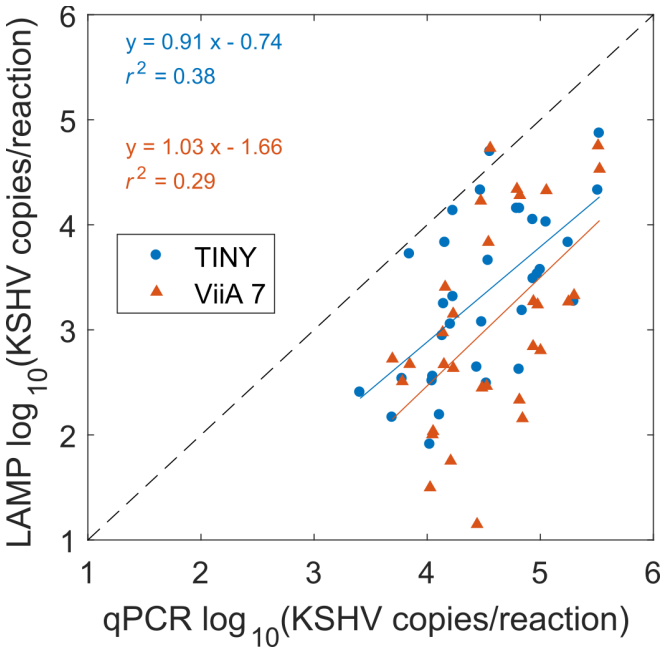
Supplementary Fig. 6. LAMP amplification efficiency is affected by sample type.

Three different sample types were tested via the LAMP assay in TINY. Two types were samples for standard curve preparation (plasmid DNA and BC-3 cell line DNA), and the third sample type was the extracted DNA from human skin biopsy samples. The true concentration of all three sample types was determined via qPCR. When amplified via LAMP, a difference in efficiency was observed for the three sample types. That is, although all three sample types had similar KSHV concentrations (as determined by qPCR), threshold time from the LAMP assay was not consistent between sample types. Plasmid DNA standards amplified the most efficiently, followed by BC-3 cell line standards. Human biopsy samples amplified the least efficiently. The dashed blue line is a best fit of the 33 human samples analyzed in the US with detectable amounts of KSHV (as determined by LAMP in TINY). The discrepancy in amplification efficiency may be explained by sample composition and/or extraction procedure used. DNA from the BC-3 cell line samples was extracted using the same extraction procedure as the human skin samples (DNeasy, Qiagen). The lower amplification efficiency when amplifying human samples explains why the quantification of those samples via LAMP is lower than when quantified via qPCR. Plotted: mean ± standard deviation.



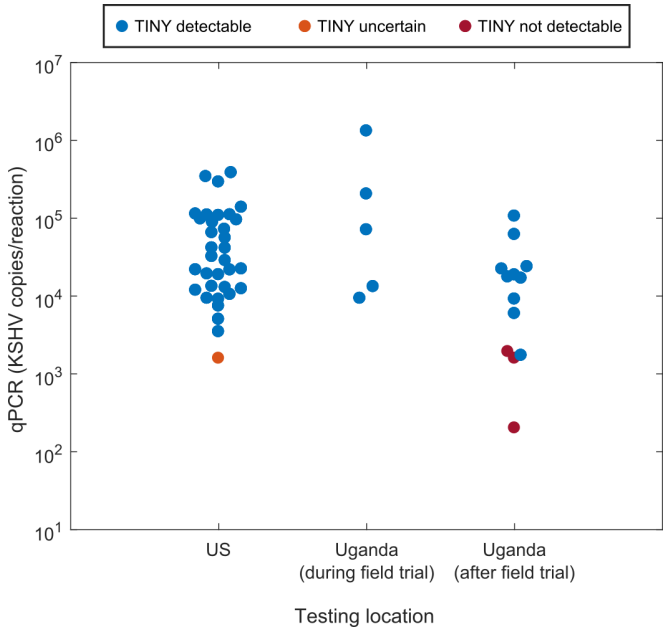
Supplementary Fig. 7. Difference between qPCR and LAMP quantification.

The difference in quantification between the two assays is reported in orders of magnitude of copies/reaction. Only the 33 human samples with detectable amounts of KSHV (as determined by LAMP in TINY) are considered in this figure.



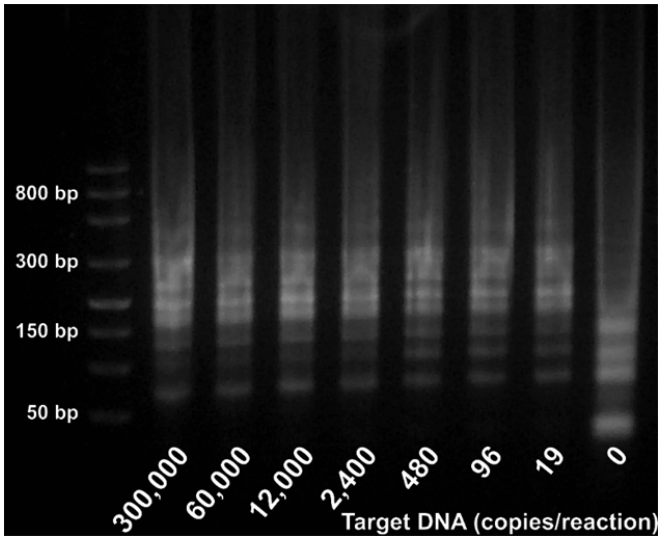
Supplementary Fig. 8. ViiA 7 quantification of human skin samples.

Quantification of skin samples for KSHV DNA by LAMP performed in the ViiA 7 commercial machine. For comparison, quantification by LAMP performed in TINY is also included. 33 of 42 samples amplified in TINY in under 24 minutes for both trials, while 31 of 42 samples amplified in the ViiA 7 in under 24 minutes for both trials (these 31 were also positive for KSHV DNA in TINY). Two replicates were performed in each machine for each patient sample, and all replicates agreed for KSHV DNA presence except for one sample in TINY (Fig. 6a) and three samples in the ViiA 7. Dashed line represents where quantification from LAMP and qPCR agree perfectly. r^2 is the ordinary coefficient of determination.



Supplementary Fig. 9. All samples with KSHV DNA sorted by TINY result and location tested.

Sorting the patients by KSHV concentration (as determined by qPCR) reveals that the four samples with discordant TINY result were among those with the lowest KSHV DNA concentration tested at any of the locations (either US or Uganda). The lower TINY-qPCR agreement for the samples tested in Uganda after the field trial (86%) as compared to the samples tested in the US (98%) can be explained by the observation that the latter samples were from patients with higher true KSHV DNA concentration.



Supplementary Fig. 10. Gel electrophoresis of LAMP products.

Plasmid samples of differing KSHV DNA concentration were amplified in TINY for 50 minutes.

Supplementary tables

Supplementary Table 1. TINY compared to other portable systems for isothermal NAT.

Device	Isothermal assay	Real-time, quantitative?	Samples per test	Consumable	Heat source	Heating resilient to unreliable electricity?	Validated in resource limited setting?	Validated using human samples?
TINY	LAMP	Yes	6	PCR tube	Electricity, sunlight, flame	Yes	Yes, Uganda	Skin biopsies
LaBarre et al. PLOS ONE 2011	LAMP	No	3	PCR tube	Exothermic reaction (CaO)	N/A	No	No
Singleton et al. PLOS ONE 2014	LAMP	No	4	PCR tube	Exothermic reaction (MgFe)	N/A	No	Spiked plasma
Curtis et al. J. Virol. Methods 2016	LAMP	No	3	PCR tube	Exothermic reaction (MgFe)	N/A	No	Whole blood
Song et al. Anal. Chem. 2016	LAMP	No	4	Microfluidic chip	Exothermic reaction (MgFe)	N/A	No	Spiked saliva
Liao et al. Sens. Actuators B 2016	LAMP	Yes	3	Microfluidic chip	Exothermic reaction (MgFe)	N/A	No	No
Craw et al. Sensors 2015	HDA	Yes	6	Microfluidic chip	Electricity	No	No	No
Stedtfeld et al. Lab. Chip 2012	LAMP	Yes	4	Microfluidic chip	Electricity	No	No	No

Supplementary Table 2. Energy requirement analysis for TINY.

Heat TINY from 23 to 68°C

Part	Material	Volume (mL)	Density (g/mL)	Heat capacity (J/g*C)	Heat (J)
Inner cylinder	Al	18.21	2.7	0.896	1982
PCR tube holder	Al	13.63	2.7	0.896	1484
Photodiode holder	Al	6.82	2.7	0.896	742
Lid	Al	22.14	2.7	0.896	2410
Outer cylinder	Al	53.45	2.7	0.896	5819
PCM	Puretemp 68	75.92	0.87	1.85	5499

Melt phase change material

Part	Material	Volume (mL)	Density (g/mL)	Heat of fusion (J/g)	Heat (J)
PCM	Puretemp 68	75.92	0.87	213	14069

Operate TINY microcontroller for one hour

Average current (A)	Voltage (V)	Time (s)	Energy (J)
0.055	5	3600	990

Energy requirement summary

Contribution	Energy (J)	% of total
Heat aluminum	12438	38
Heat PCM	5499	17
Melt PCM	14069	43
Operate microcontroller	990	3
TINY total	32995	100

Supplementary Table 3. Standard curve data for LAMP and qPCR assays.

		Threshold cycle or threshold time in minutes										
Sample name	**Concentration (copies/reaction)**	**LAMP in TINY trials**					**LAMP in ViiA 7 trials**				**qPCR trials**	
Plasmid-A	300000	9.67	9.92	10.08	9.83	9.75	9.19	9.14	-	-	19.35	19.04
Plasmid-B	60000	11.08	10.25	11.17	11.08	10.58	10.26	10.06	-	-	21.62	21.45
Plasmid-C	12000	11.50	11.33	12.08	11.58	12.00	11.19	10.68	-	-	23.89	23.83
Plasmid-D	2400	12.50	13.25	12.67	12.42	13.17	12.88	12.64	-	-	26.33	26.00
Plasmid-E	480	16.83	15.25	15.08	14.33	17.50	15.02	12.55	-	-	28.59	28.47
Plasmid-F	96	20.25	16.83	20.67	17.92	18.58	12.29	13.70	-	-	30.74	30.64
Plasmid-G	19	31.17	17.42	15.83	47.67	19.42	NA	18.72	-	-	32.81	32.79
Plasmid-H	0 (NC)	NA	NA	NA	NA	NA	26.44	NA	-	-	NA	37.32
BC3-1	394440	10.92	11.24	10.53	11.24	11.12	11.03	11.47	11.26	11.02	22.61	22.38
BC3-2	90426	11.83	12.83	11.92	12.50	11.83	11.80	12.36	11.71	11.87	24.49	24.44
BC3-3	14270	13.50	13.67	13.67	13.17	13.00	13.60	13.48	13.17	12.68	26.86	27.00
BC3-4	3266	15.67	15.42	16.17	13.58	14.92	14.42	14.87	15.41	14.50	28.88	28.93
BC3-5	708	18.92	16.17	19.83	16.42	-	18.87	16.75	16.06	17.94	30.99	30.91
BC3-6	135	18.92	20.42	19.75	19.33	-	23.23	19.59	21.18	28.64	33.10	33.24
BC3-7	19	31.67	21.50	20.33	43.00	-	25.27	22.73	NA	21.53	35.94	35.64
BC3-8	0 (NC)	NA	NA	NA	NA	-	25.52	26.59	24.40	23.97	NA	NA

Key: "-" Trial not performed

"NA" No amplification

Supplementary Table 4. Primer sequences for PCR and LAMP assays.

Primer	Length (bp)	Target	Sequence (5' to 3')
PCR-Forward	19	KSHV/ORF 26	AGCCGAAAGGATTCCACCA
PCR-Reverse	16	KSHV/ORF 26	GCTGCGGCACGACCAT
LAMP-FIP	41	KSHV/ORF 26	TGGATTCGAGCACAATGGTGGACAACACCCAGC TAGCAGTG
LAMP-BIP	40	KSHV/ORF 26	TCGTGTTCCCCATGGTCGTGAGATGTGGTACACC AACAGC
LAMP-B3	19	KSHV/ORF 26	CCGGCCGATATTTTGGAGT
LAMP-F3	19	KSHV/ORF 26	TGCCCCCTTTTTTCAGTGG
LAMP-LOOPF	25	KSHV/ORF 26	CCTTTCGGCTAAAAAATGGGGGTAG
LAMP-LOOPB	20	KSHV/ORF 26	CAGCAACTGGGGCACGCTAT

Sequences for LAMP primers were obtained from:

T. Kuhara et al., Rapid detection of human herpesvirus 8 DNA using loop-mediated isothermal amplification. J. Virol. Methods. 144, 79–85 (2007).