A portable device for nucleic acid quantification powered by sunlight, a flame or electricity

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A decentralized approach to diagnostics can decrease the time to treatment of infectious diseases in resource-limited settings, yet most modern diagnostic tools require stable electricity and are not portable. Here, we describe a portable device for isothermal nucleic acid quantification that can operate with power from electricity, sunlight or a flame, and that can store heat from intermittent energy sources for operation when electrical power is not available or reliable. We deployed the device in two Ugandan health clinics, where it successfully operated through multiple power outages, with equivalent performance when powered via sunlight or electricity. A direct comparison between the portable device and commercial quantitative polymerase chain reaction machines for samples from 71 Ugandan patients (29 of which were tested in Uganda) for the presence of Kaposi’s sarcoma-associated herpesvirus DNA showed 94% agreement, with the four discordant samples having the lowest concentration of the herpesvirus DNA. The device’s flexibility in power supply provides a needed solution for on-field diagnostics.

Communicable diseases such as human immunodeficiency virus (HIV) infection, malaria and respiratory infections are among the leading causes of death in low-income countries. While treatment for many infectious diseases is available worldwide, effective and widespread diagnosis remains a challenge. For example, a nucleic acid test (NAT) is required for early infancy diagnosis of HIV, but in 2014 only half of the estimated 1.2 million infants exposed to HIV received a diagnostic test. Furthermore, NATs that are quantitative are required for applications such as HIV viral load monitoring, but such tests are still largely unavailable in the resource-limited settings where infectious diseases are most common.

Traditional diagnostics in low- and middle-income countries (LMIC) may be burdened by lengthy procedures for transporting human samples from rural healthcare clinics to central laboratories. Modern tools have aimed to disrupt this dependency on centralized laboratories to improve the time to treatment of infectious diseases. For example, tuberculosis time to treatment in Cape Town, South Africa was decreased from 71 days (centralized) to 8 days (decentralized) following implementation of the GeneXpert. The GeneXpert (Cepheid) is a fully automated system for NATs and has reported good clinical performance; however, the GeneXpert IV is not portable, has an instrument cost of about US$17,000 (ref. 16) and requires a dedicated electricity supply (Supplementary Fig. 1).

Electricity dependence is a critical issue for using such tools in LMIC: in 11 sub-Saharan African countries, one-fourth of healthcare facilities lack access to reliable electricity. Furthermore, in populations that are largely rural—for instance, 75% of Ugandan households are rural—systems for NATs should be portable, enabling transportation between clinics.

Because they negate the need for thermal cycling, many forms of isothermal nucleic acid amplification (LAMP) is one such isothermal method, and is capable of nucleic acid quantification. Simple systems for performing isothermal amplification in resource-limited settings exist, although many are only qualitative, and those that are quantitative use microfluidic chips as consumables, often making them impractical to use in the field. For heat input, these systems either use exothermic chemical reaction packets, or stable electricity. None have the flexibility to use electricity when it is available, and alternative heat sources when electricity is unavailable (Supplementary Table 1).

Here, we present a portable device called Tiny Isothermal Nucleic acid quantification sYstem (TINY). TINY can be heated from a variety of energy sources, including sunlight, flame, or electricity, giving it the unique capability to be operated in a laboratory when electricity is available, or in the field when electricity is unavailable. TINY enables nucleic acid quantification in a handheld package (Fig. 1a), and its weight and volume are approximately an order of magnitude smaller when compared with commercial quantitative polymerase chain reaction (qPCR) machines (Fig. 1b). TINY can use a variety of heat sources (Fig. 1c–e) because it stores heat isothermally through use of a phase-change material (PCM); thermal cycling is not required as TINY performs LAMP. The latent heat of the melted PCM inside TINY keeps the system isothermal for over an hour in case of power outages when heated by electricity, or in case of variable cloud coverage when heated via sunlight.

After describing how TINY functions, we evaluate the system against commercial machines performing both qPCR and LAMP. The evaluation is conducted on human skin biopsies from Ugandan patients suspected of Kaposi’s sarcoma (KS). Kaposi’s sarcoma is caused by the Kaposi’s sarcoma-associated herpesvirus (KSHV, also formally known as human herpesvirus 8), and is most common in HIV-infected individuals. Diagnosis of KS via NAT for KSHV DNA in skin lesions is being considered as an alternative to current diagnostics (visual inspection or histology) because the accuracy of those methods in LMIC is often low. Our results suggest that

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TINY performs the LAMP assay with accuracy equivalent to commercial machines. However, we did find that LAMP underperforms qPCR when quantification is desired. We also deployed TINY at two Ugandan health clinics in November 2017, and we report comparable performance in the field as in the laboratory. Four months following TINY deployment, Ugandan staff analysed additional patients and were able to independently obtain results that mostly agreed with gold-standard qPCR performed in the US.

Results

TINY design and construction. TINY is built from two units performing separate functions. A temperature-regulation unit is responsible for heat collection and isothermal stabilization. A measurement unit is responsible for tracking the progress of the NAT. A picture of the measurement unit is shown in Fig. 2a, and a cross-section view in Fig. 2b. It is made from aluminium and contains six wells to insert samples into. PCR tubes, 0.2 ml in size, are used as plastic consumables, as they are inexpensive and easily accessible. Printed circuit boards (PCBs) mounted to the top and bottom of the measurement unit hold the optical sensors for monitoring the LAMP reaction. Light-emitting diodes (LEDs) affixed to the top PCB excite commonly used fluorophores in the sample (Fig. 2c). A dual bandpass optical filter is placed above photodiodes on the bottom PCB, allowing TINY to measure both fluorescence and absorbance by cycling the active LED.

The measurement unit is placed into the centre of the temperature-regulation unit (Fig. 2d), which is made from concentric aluminium cylinders. PCM is inserted between the two cylinders: PureTemp 68 is used because its melting temperature (68 °C) is suitable for the LAMP reaction. The PCM serves two functions. First, it acts as a thermal buffer to make sure that the temperature of the samples does not get too high: heat input may be attenuated before temperature increase begins after the melting stage. Second, it serves as a large heat reservoir for operation with unreliable heat sources. For example, solar energy may be collected in excess when available and stored in the form of latent heat, allowing for isothermal operation even if clouds block the Sun during LAMP. The volume of required PCM was estimated via COMSOL simulation (Supplementary Fig. 2), where our goal was >1 h of dwell time at 68 ± 1 °C in the case of total heat source disruption.

When assembled inside an aluminium enclosure, the volume and weight of TINY is 2.1 l and 1.1 kg, respectively. TINY can accept heat from both the top and bottom of the outer aluminium cylinder (Fig. 2e). If heating via sunlight, a Fresnel lens is used to concentrate sunlight onto an absorber plate (Fig. 1c). A supporting structure allows a user to rotate the lens for alignment with the Sun, and we found that lens readjustment was necessary between 1–3 times when heating TINY via sunlight (depending on location and time of year). At present, TINY is designed to operate at solar altitude angles >50°.

Isothermal for 65 min following heat disruption. Two of the heat sources available for operating TINY are electricity and sunlight. To be resistant to electricity outages and cloud coverage, TINY stores a large amount of heat (14 kJ) in the latent heat of a PCM. Even in cases of complete heat source disruption, this heat storage enables TINY to stay isothermal for about 65 min (Fig. 3a)—sufficient time for about two LAMP reactions. The temperature stability provided by the PCM is illustrated well when compared with water: we replaced the PCM in TINY with water and found that the system stayed isothermal for only 11% as long (Fig. 3b). If stable electricity is available, the system stays isothermal indefinitely (Fig. 3c).

While the heating of TINY need not be provided by electricity, electricity is required to power TINY’s sensors. Only a small amount (3%) of TINY’s total energy requirement is electrical (Fig. 3d and Supplementary Table 2). Therefore, TINY is uniquely suitable for operation in resource-limited settings because most of the required energy (heating) can be supplied via sunlight or flame. TINY can operate in the field permanently using solar thermal heating and a small photovoltaic cell to power the electronics, while systems that rely solely on batteries for field use cannot (Supplementary Fig. 3). Extended field operation is also possible without photovoltaics; for...
example, an iPhone 6s battery (capacity, 6.9 Wh) can power TINY’s electronics for over 24 h.

**LAMP assay in TINY is independent of heat source.** We heated TINY using a variety of heat sources, with the hypothesis that all heat sources would be able to reach the isothermal condition desired for the LAMP reaction. Figure 3e shows temperature profiles of TINY during heat-up using a Bunsen burner, a small hotplate and sunlight. We found that heating TINY for about half an hour in sunlight was sufficient to melt all the PCM and to sustain the long isothermal dwell, although this is dependent on ambient conditions. Once while collecting sunlight, TINY experienced complete cloud coverage for about 6 min, but the effect of the cloud was to only delay heating of TINY (Fig. 3f). In contrast, a previously developed microfluidic device that performed PCR via solar thermal heating is only capable of operation during clear-sky operation34,35.

We hypothesized that TINY would perform the LAMP assay equivalently using any of the heating methods. LAMP reactions were performed when TINY was heated by a hotplate, a Bunsen burner and by sunlight. The average sample temperature for each of these experiments was just above 68 °C, and only deviated by 0.3 °C between the heating methods (Fig. 4a). Similar threshold times were observed when the same sample was amplified in TINY, no matter the heating method (Fig. 4b). Threshold times were calculated by tracking fluorescence data in real time (Fig. 4c, and Supplementary Figs. 4 and 5).

**Standard curves show TINY provides comparable quantification.** We evaluated TINY’s capability to perform quantitative NATs using skin biopsy samples from patients suspected of KS. To quantify KSHV load in unknown-concentration skin biopsy samples, standard curves with known copy numbers of the KS target gene, **ORF 26**, were generated from recombinant plasmid DNA, and DNA extracted from a KSHV+ cell line, BC-3 (ref. 36).

The following observations are drawn from the KSHV+ cell line (BC-3) standards, as the DNA in these samples was extracted using the same procedure as for the human biopsy samples. The qPCR assay proved quantitative for all concentrations of standards (Fig. 5a). The LAMP assay produced repeatable threshold times for the four highest standards tested (3.2 × 10^4 to 3.9 × 10^5 copies per reaction), but at lower concentrations, threshold time no longer linearly predicted starting DNA concentration. At the lowest concentration (19 copies per reaction), the LAMP assay amplified in 7 of 8 trials, and at the second lowest concentration (135 copies per reaction), the LAMP assay amplified in 8 of 8 trials. A 2007 study using a similar assay found a limit of detection of approximately 100 copies per reaction37. We also observed that the amplification efficiency of the LAMP assay was dependent on the type of sample being amplified (Supplementary Fig. 6 and Supplementary Table 3).

We amplified standard samples using LAMP in both TINY and a commercial qPCR machine (ViiA 7, Thermo Fisher Scientific, set to 68 °C). Similar standard curves were produced using both machines (Fig. 5b), confirming that TINY can perform quantitative,
isothermal assays with results that are equivalent to those from commercial systems.

**Human skin biopsies analysed by TINY and commercial machines.** We collected human biopsy samples from 42 Ugandan patients suspected of having KS, and tested these samples via LAMP in TINY, via LAMP in the Viia 7, and via traditional qPCR in an Applied Biosystems 7500 Fast. Samples were collected at the Infectious Diseases Institute (IDI) of Makerere University (Kampala, Uganda), and then transferred to the US for analysis.

TINY–qPCR agreement was 41/42 (98%) on a binary, detectable/not-detectable basis, with both systems finding the same 8 patients negative (Fig. 6a). For the sample with the lowest KSHV concentration, TINY gave a mixed positive/negative result (samples were tested twice using each system/assay). We note that the diagnostic value of this analysis cannot be assessed without histological confirmation and a larger sample size.

Next, the 33 samples with TINY-detectable KSHV levels were analysed quantitatively. We compared quantification by LAMP (performed in TINY), finding a coefficient of determination $r^2 = 0.48$ (Fig. 6b). A similar coefficient of determination ($r^2 = 0.38$) was found in a previous study that compared LAMP and qPCR quantification. In all cases except for one, we observed that the quantification obtained from the LAMP assay was lower than the quantification obtained from the qPCR assay (Supplementary Fig. 7). This observation has been
often disagree in quantification by an order of magnitude or more with high reproducibility, while replicate trials of LAMP could quantify the same sample multiple times, as a machine.

Sample temperatures were measured and reported as shown in Fig. 4. Each experiment was performed once, but similar profiles are obtained when TINY is cooled in a room-temperature environment from a fully heated state. The threshold times of samples containing the same target DNA concentration (12,000 copies per reaction) but heated using different sources. The average time of four samples is displayed above each method. The data are from the same experiments shown in Fig. 2. Measurement resolution was 5 s. c. The fluorescent signal measured in TINY during nucleic acid amplification. The threshold time (large data points) is taken as the time the fluorescence passes a predefined threshold. Samples were inserted into TINY at 0 min. Samples were inserted into TINY at 0 min. Each experiment was performed once, but similar profiles are obtained when TINY is cooled in a room-temperature environment from a fully heated state. The threshold times of samples containing the same target DNA concentration (12,000 copies per reaction) but heated using different sources. The average time of four samples is displayed above each method. The data are from the same experiments shown in Fig. 2. Measurement resolution was 5 s. c. The fluorescent signal measured in TINY during nucleic acid amplification. The threshold time (large data points) is taken as the time the fluorescence passes a predefined threshold. Samples were inserted into TINY at 0 min. Fluorescence curves are demonstrative and show the response from TINY’s optical sensors from a single experiment.

Previously reported in a study comparing digital LAMP and digital PCR. Quantification of the human samples via LAMP was similar whether performed in TINY or the ViiA 7 commercial machine. Three separate LAMP reactions, each with a different heating method. Average temperatures (T) are reported in the top-right corner. Samples were inserted into TINY at 0 min. Each experiment was performed once, but similar profiles are obtained when TINY is cooled in a room-temperature environment from a fully heated state. The threshold times of samples containing the same target DNA concentration (12,000 copies per reaction) but heated using different sources. The average time of four samples is displayed above each method. The data are from the same experiments shown in Fig. 2. Measurement resolution was 5 s. c. The fluorescent signal measured in TINY during nucleic acid amplification. The threshold time (large data points) is taken as the time the fluorescence passes a predefined threshold. Samples were inserted into TINY at 0 min. Fluorescence curves are demonstrative and show the response from TINY’s optical sensors from a single experiment.

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TINY evaluated at Ugandan health clinics. In 2017, we conducted a field trial of the TINY system in partnership with two Ugandan health clinics that regularly diagnose KS-suspect patients using visual inspection and/or histology. The field trial took place at the IDI in Kampala, and the AIDS Healthcare Foundation—Uganda Cares Clinic in Masaka.

One of the goals of this effort was to characterize the sample-to-answer timeline and to demonstrate that results from TINY could be obtained on a clinically relevant timescale. Three KS-suspect patients presented at the clinics during our field trial. Biopsies were collected from the patients and a portion of each biopsy was immediately used for DNA extraction and subsequent analysis in TINY. Results from TINY were obtained about 2.5 h following the start of the biopsy procedure (Fig. 7a). DNA extraction was the longest part of the process (85 min on average).

We hypothesized that results from TINY would not depend on the location of the test (US versus Uganda), the heating method used (electricity versus sunlight), or the device operator (TINY developers versus local staff). DNA was extracted from 8 KS-suspect biopsies at the IDI in Uganda and was amplified under different experimental conditions. We found that the same 5 samples were positive for KSHV DNA regardless of the location, heating method, or device operator for TINY (Table 1), including samples amplified using sunlight (Fig. 7b). The threshold times for these 8 samples were similar across a large variety of conditions (Fig. 7c), even when switching from liquid to lyophilized reagents (for applications where maintaining the cold chain is not feasible). Furthermore, when threshold times were grouped by patient, the resulting clustering showed that quantification by TINY is possible across all locations and heating methods, as the variation in threshold time (meaning that differences in threshold time of a few minutes can occur from assay variation and not only because of differences in target nucleic acid concentration). The difference in quantification was similar for both TINY and the ViiA 7, further confirming that the correlation between LAMP and qPCR quantification (Fig. 6b) is a result of the assays and not the machine used. We considered quantifying samples using either fluorescence or absorbance data from TINY, and found the two methods equally capable (Fig. 6d).
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TINY in Uganda several months after deployment. Here, we have found that one of the greatest assets of TINY was its usability in the field. Our team has previously developed microfluidic devices for NATs in resource-limited settings, particularly for use in Uganda. TINY—because it uses off-the-shelf, inexpensive consumables, and because it does not require any pumps or tubing—was much easier to operate in the field than the microfluidic devices for NATs in resource-limited settings, particularly for use in the laboratory after heating, TINY stayed within our target temperature ±1 °C target temperature, regardless of heating method or electricity outages (Fig. 7e). We performed 19, 6-sample experiments (114 samples) during the 5 day field trial. After training the local staff how to operate TINY, they were proficient at operating the system autonomously, and they obtained the same results for the 8 samples as did the Cornell team (Table 1).

Four months after the field trial, human biopsies from 21 new patients were analysed in TINY at the IDI in Uganda. DNA extraction and TINY operation was completed by the Ugandan team without help from TINY developers except for the instruction manuals left during the field trial. Four different individuals performed LAMP using TINY during this time. Of the 21 patients, qPCR performed in the US determined 8 to be negative for KSHV DNA, and 13 to be positive. TINY performed in Uganda agreed with qPCR on a binary level for 18 of 21 patients (86%) (Fig. 7f). The concentrations of the three discordant samples were among the three lowest of 13 to be positive. TINY performed in Uganda agreed with qPCR on a binary level for 18 of 21 patients (86%) (Fig. 7f).

Discussion

We found that one of the greatest assets of TINY was its usability in the field. Our team has previously developed microfluidic devices for NATs in resource-limited settings, particularly for use in Uganda. TINY—because it uses off-the-shelf, inexpensive consumables, and because it does not require any pumps or tubing—was much easier to operate in the field than the microfluidic device. This observation is supported by the successful operation of TINY in Uganda several months after deployment. Here, we have compared TINY with both commercial and research-grade systems for NATs (Supplementary Table 1). TINY is the only system that can use electrical and non-electrical energy sources, making it uniquely suited for extended operation both in the laboratory and in the field, even when compared with battery-powered commercial systems. Our results show that quantification by TINY is on par with commercial systems performing the same assay, meaning that diagnostic performance need not be sacrificed for system portability.

We validated TINY by testing human skin samples from Uganda for KSHV DNA. A total of 71 patient samples were analysed (42 in the US, 8 in Uganda during the field trial and 21 more in Uganda after the field trial). TINY–qPCR agreement was 67/71 (94%) across all patients, and the four discordant specimens (all false negatives) were among the four samples with lowest KSHV concentrations (Supplementary Fig. 9), suggesting that the disagreement resulted from a lack of assay sensitivity and not TINY capability. Compared with qPCR, we found that the LAMP assay was inferior in its ability to quantify nucleic acids at low concentrations (as found by other studies), and that the repeatability of quantification for the same sample was relatively low. We showed that mediocre quantification repeatability was a result of the LAMP assay and not a result of the TINY itself (Fig. 6c and Supplementary Fig. 8) or of the heating method itself (Fig. 6c and Supplementary Fig. 8) or of the heating method used (Fig. 4b and Fig. 7d). While LAMP underperformed qPCR, our work did not focus on optimization of the assay, and improvements could be implemented: for example, stabilization of LAMP via chemical additives, and that the repeatability of quantification for the same sample was relatively low. We showed that mediocre quantification repeatability was a result of the LAMP assay and not a result of the TINY itself (Fig. 6c and Supplementary Fig. 8) or of the heating method used (Fig. 4b and Fig. 7d).

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Our field trial in Uganda confirms that TINY is particularly useful for operation in resource-limited settings. The small size of TINY made it convenient to transport to two Ugandan clinics, and TINY results for KSHV DNA were consistent using a variety of locations, device operators and heating conditions, including sunlight. TINY successfully completed multiple LAMP reactions even though electricity outages were experienced mid-assay. During the outages that TINY successfully operated through, commercial machines running diagnostics in the same laboratory had their assays ruined, even though...
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a generator and backup batteries were installed for such situations (generator failed to start). Several KS-suspect patients arrived at the Ugandan clinics during our field trial, and we were able to obtain TINY results in just 2.5 h following the start of the biopsy procedure. Four months after the field trial, the Ugandan team independently analysed 21 more samples in TINY with strong agreement to gold-standard qPCR performed in the US.

Although we used TINY to perform LAMP, replacement of the PCM with one that melts at other temperatures would allow the system to perform other isothermal assays, making the system broadly useful. TINY is suitable for multiple applications in LMIC. For example, TINY could be carried by healthcare workers traveling between communities, providing diagnostics to patients unable to travel to urban healthcare institutions. TINY could also be used as a stationary tool in district-level clinics and hospitals, where its unique ability to use unreliable electricity would be of value. Both applications can enable nucleic acid diagnostics to reach more of the population in LMIC.

Methods

LAMP assay composition. LAMP uses a strand displacement polymerase and a set of four to six DNA primers to create amplicons that resemble cauliflower-like, stem-loop DNA structures in less than an hour13. Our LAMP assay contained 320 U ml−1 of Bst 2.0 WarmStart DNA Polymerase, 1× isothermal amplification buffer, 6 mM MgSO4, 1.4 mM dNTP mix (all from New England BioLabs Inc.), along with primers: 1.6 μM FIP/BIP, 0.2 μM F3/B3 and 0.4 μM LoopF/LoopB. Isothermal primers were designed previously37 with ORF26 as the target (Supplementary Table 4). We also added Evagreen fluorescent dye (Biotium) to final concentration 1×, and ROX reference dye (Thermo Fisher Scientific) to final concentration 2×. LAMP amplicons were confirmed via gel electrophoresis (Supplementary Fig. 10).

Sample preparation for amplification in TINY and Viia7. Four millilitres of master mix was made before performing quantification experiments. This mix was aliquoted into tubes for individual experiments to be performed in TINY, and then frozen. The large master mix was made to minimize variation in assay composition that might arise from pipetting errors during the preparation of multiple master mixes, so that threshold times could be compared between experiments. The master mix contained all reagents except for Bst 2.0 WarmStart polymerase, nuclease-free water and DNA sample. To prepare a sample for amplification in...
TINY, Bst 2.0 Warmstart DNA Polymerase and water were added to the master mix, and then 35 µl of this mixture was aliquoted into a PCR tube. Next, 5 µl of DNA sample was added to the PCR tube and mixed by repeated pipetting. Finally, 50 µl of paraffin oil was placed on top of the LAMP assay to prevent evaporation. For amplifications performed in the ViiA 7 qPCR machine, the same assay was used except 2.5 µl of DNA sample and 17.5 µl of the mixture containing all other reagents were combined in individual wells in a 96-well qPCR plate. No oil was used for ViiA 7 amplifications.

Isothermal amplification in TINY. All nucleic acid amplification experiments in TINY started with heating the system to 97 °C. If too much heat was put into the system, the inner system temperature (sample temperature) was cooled to at least 70 °C before beginning LAMP. When the temperature was suitable for amplification, we removed the lid of TINY, inserted the PCR tubes into sample holes and replaced the lid. A microcontroller (Teensy 3.2) running an Arduino programme was used to track the temperature, fluorescence and absorbance of the samples throughout the course of the LAMP reaction (at least 50 min). Sampling times between 95 °C for 3 s and 60 °C for 30 s. Each reaction of the ViiA 7 was thermal-cycled with holding at 95 °C for 20 s before cycling 40 times between 95 °C for 2 s and 60 °C for 1 min. All samples were run in duplicate against a standard curve to estimate copy number. Resulting BC-3 standards used in LAMP amplified linearly via qPCR and were estimated to contain copy numbers on the same order of magnitude as the plasmid standard curve.

DNA extraction from human samples. All ethical regulations were complied with during this study. Written, informed consent was obtained for all patients involved. The study was approved by the Makerere University School of Biomedical Sciences Ethics Review Committee.

Cylindrical (4 mm diameter) punch biopsies of skin lesions were obtained from Ugandan adults who had at least some level of clinical suspicion for KS and who were referred to the IDI in Kampala for a diagnostic biopsy. Biopsies were stored in RNAlater (Qiagen, cat. no. 76104) and later bisected. Half of the biopsy was processed using the purification of Total DNA from Animal Tissues protocol of the DNeasy Blood & Tissue kit (Qiagen, cat. no. 69504) and resulting DNA was eluted in 75 µl of Buffer AE. Total DNA concentration and purity were assessed for each sample via NanoDrop spectrophotometry.

qPCR assay. TaqMan assays were used for real-time amplification and detection of viral ORF26 and control gene GAPDH in qPCR. Each reaction of the custom ORF26 assay was performed at a total volume of 20 µl containing: 10 µl of PrimeTime Gene Expression Master Mix (IDT, cat. no. 1055770), 1.8 µl of a 10 µM forward and reverse primer mix (primer sequences in Supplementary Table 4), 2.2 µl nuclelease-free water, 1 µl of 5 µM ORF 26 probe, and 5 µl of sample. The ORF 26 assay was thermal-cycled with holding at 95 °C for 20 s before cycling 40 times between 95 °C for 2 s and 60 °C for 30 s. Each reaction of the GAPDH assay was performed at a total reaction volume of 10 µl containing: 5 µl of TaqMan Genotyping Master Mix (Thermo Scientific, cat. no. 4371355), 0.5 µl of a 20X GAPDH TaqMan Copy Number Assay (Thermo Scientific, cat. no. 4400292- H000483111_c1), and 4.5 µl of sample. The GAPDH assay was thermal-cycled with holding at 50°C for 2 min, 95°C for 10 min, then cycling 40 times between 95°C for 15 s and 60°C for 1 min. All samples were run in duplicate against a standard plasmid curve. Late threshold cycle (C) values outside the range of the standard curve were considered inconclusive/negative. Raw tissue biopsy DNA extracts were run directly as the assay input and verified with standard 10 ng dilutions in both assays. All samples showed high copy number of GAPDH.

Table 1 | TINY results for eight human samples tested in Uganda

| Patient | IDI interrupted electricity | IDI electricity | IDI sunlight | IDI electricity, yohiophilized reagents | IDI electricity, operated by Uganda staff | Masaka electricity | Masaka sunlight | Cornell (Ithaca) | LAMP+ | qPCR |
|---------|-----------------------------|----------------|--------------|----------------------------------------|------------------------------------------|------------------|----------------|----------------|-------|------|
| A       | +                           | +              | +            | +                                      | +                                        | +                | +              | +              | 2.64  | 4.12 |
| B       | +                           | +              | +            | +                                      | +                                        | +                | +              | 4.02           | 5.31  |
| C       | Power outage not experienced (patients C-H) | + | + | + | + | + | + | 2.96 | 4.85 |
| D       | -                           | -              | -            | -                                      | -                                        | -                | -              | -              | -     | -    |
| E       | +                           | +              | +            | +                                      | Sunlight not available (patients E-H)   | +                | +              | 4.24           | 6.12  |
| F       | -                           | +              | +            | +                                      | -                                        | -                | -              | 4.13           | 3.97  |
| G       | -                           | -              | -            | -                                      | -                                        | -                | -              | -              | -     | -    |
| H       | -                           | -              | -            | -                                      | -                                        | -                | -              | -              | -     | -    |

DNA was extracted from biopsy samples in Uganda and then amplified in TINY at the Infectious Diseases Institute (IDI) in Kampala, or the Uganda CARES Clinic in Masaka, under a variety of test conditions. Results were confirmed at Cornell in Ithaca, New York (via LAMP) and in New York City, New York (via qPCR). No quantification is reported for patients D, G and H as no amplification was observed for these samples. Based on mean threshold time from all locations, heating methods and other test conditions.

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Author contributions
R.S. and A.G. wrote the manuscript with review from all other authors. R.S., V.K., J.D., R.S. and A.G. wrote the manuscript with review from all other authors. R.S., V.K., J.D., R.S. and A.G. analysed the data. R.S. generated the figures and tables.

Competing interests
The authors have submitted a patent for the TINY system.

Additional information
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Experimental design

1. Sample size
   Describe how sample size was determined.

   Sample size was determined by the availability of human samples (we tested all samples available at the time of measurements). Initially, 42 human samples were available and analysed in controlled laboratory conditions in the U.S. During our field trial an additional 8 samples became available, which were then analysed in Uganda. A further 21 human samples were analysed in Uganda four months after the field trial. The total sample size (71 patients) is sufficient for this study because the study focuses on the validation of TINY on a device-level and because we make no clinical claims about Kaposi’s sarcoma diagnosis using nucleic-acid measurements.

2. Data exclusions
   Describe any data exclusions.

   No data were excluded. When comparing TINY and qPCR quantification, only the samples with detectable amounts of KSHV DNA (as determined by TINY) were considered, as otherwise a comparison was not possible.

3. Replication
   Describe the measures taken to verify the reproducibility of the experimental findings.

   Nucleic-acid quantification of human samples was completed twice by each machine (for the first 42 available human samples). For qPCR, all replicates agreed on KSHV DNA presence. TINY replicates agreed for 41/42 samples, and we categorized the sample with disagreement as ‘uncertain’ for KSHV DNA presence. For measurements in the ViiA 7, 39/42 samples agreed between replicates. These 42 samples were analysed twice more by qPCR to determine if there was a discrepancy in quantification repeatability between technical and experimental replicates.

   For the samples analysed in Uganda, we performed replications by comparing experimental conditions such as location or heating method (the number of replicates varied based on availability of experimental conditions; see Table 1). However, each sample was only analysed once in TINY for each experimental condition for the experiments conducted in Uganda. The 29 samples analysed in Uganda were also analysed against qPCR results as performed in the U.S., where qPCR was performed in duplicate for each patient sample.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.

   Samples were not grouped. In this study, we considered all human samples the same and only compared results from the various nucleic-acid testing machines.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   In this study, nucleic-acid quantification was not compared to a clinical diagnosis, so blinding is not applicable. Quantification was performed without any knowledge of the patient’s health. Quantification by TINY and qPCR was performed independently by two different scientists.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☐   | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☐   | ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐   | ☒ A statement indicating how many times each experiment was replicated |
| ☐   | ☒ The statistical test(s) used and whether they are one- or two-sided |
| ☐   | ☒ Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ☐   | ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☐   | ☒ Test values indicating whether an effect is present |
| ☐   | ☒ Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted. |
| ☐   | ☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ☐   | ☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation) |

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

MATLAB was used to analyse data and produce the figures. Standard MATLAB algorithms were used for finding means and medians, and for producing box plots. Arduino software using standard libraries was used to operate TINY. QuantStudio Real-Time PCR Software was used to determine threshold times for experiments conducted in the Viia 7, using default settings. For qPCR, threshold cycles were calculated by the AB7500 software using the software's default settings.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All reagents are commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used in this study.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The BC-3 cell line was established by the Cesarman lab, from a patient with a Kaposi’s sarcoma herpesvirus positive primary effusion lymphoma (https://www.ncbi.nlm.nih.gov/pubmed/8839859).

b. Describe the method of cell line authentication used.

The cell line was authenticated via STR profiling. BC-3 matched it’s publicly available STR profile.

c. Report whether the cell lines were tested for mycoplasma contamination.

The cell line was tested for mycoplasma contamination at the point of cell-line authentication, and monitored for mycoplasma contamination periodically in-house via PCR. Cultured cell lines used in this study were consistently negative for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

All samples were obtained from Ugandan adults who were suspected of Kaposi’s sarcoma, but whose true diagnosis was unknown. Gender and treatment status were not considered.