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Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. <u>For final submission</u>: please carefully check your responses for accuracy; you will not be able to make changes later.

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

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

scientists.

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
\boxtimes	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.
\ge	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
\boxtimes	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 <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range)

 \Box Clearly defined error bars in <u>all</u> 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.

9. Antibodies

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

- 10. Eukaryotic cell lines
 - a. State the source of each eukaryotic cell line used.
 - b. Describe the method of cell line authentication used.
 - c. Report whether the cell lines were tested 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 antibodies were used in this study.

All reagents are commercially available.

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).

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

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.

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.