EMBO reports

Scalable RT-LAMP-based SARS-CoV-2 testing for infection surveillance in pandemic preparedness

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

Dear Michael,

Thank you for submitting your manuscript on the LAMP SARS-COV-2 testing pipeline for consideration by the EMBO Reports. It has been seen by two referees whose comments are enclosed and we received encouraging informal advice from a third expert. As you will see, both referee reports require satisfactory revision before a final decision for publication can be made.

Given the referees' constructive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing all reviewer comments substantially.
In particular,
1) the importance of the manuscript rests in the application of this method in favour of canonical RT-qPCR testing. The current manuscript does not provide a compelling economic, resource and time analysis of both methods to make such a case. We concur with both referees 1 and 2 that this has to be added. Related to this, re. p25: please add information on the % of success with the RT-qPCR validation to gauge performance better. Instead, remove the unfounded speed claim on p.25 (cf. ref 1).
2) The second important point is to discuss the viability of applying this beyond a migh infrastructure support academic setting (again in comparison with RT-qPCR); see also ref 1.
3) Reduce the text where it becomes anecdotal, in particular in the discussion (cf. ref 1). For example where you discuss times of day for testing; 'due to storage limitations...'; Roehr et al self citation); Instead, please add definitive argument for applying LAMP for SAR-CoV-2 or indeed other infection settings; see also ref 1.
4) improve analysis/visualiziation section and Github documentation (ref 2)

specific:
1) p25: for the time series, explain if a timeline is important or not (cf. re 1). What % of cases were 'unclear cases where a fill LAMP reaction was inspected'?
2) Add better context as suggested by ref 2.
3) address 'intrinsic limitations...without mlecular beacons' (ref 1).
4) Fig 1B: add control (or remove claim); see ref 1.
5) Fig 1D: add RT-qPCR; see ref 1.
6) Also address ref 1, figs S2D, 2C, 5B.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

best wishes,

Bernd

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Instructions for preparing your revised manuscript:

Please check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
https://bit.ly/EMBOPressFigurePreparationGuideline
See also figure legend guidelines: https://www.embopress.org/page/journal/14602075/authorguide#figureformat

IMPORTANT: When you send the revision we will require

- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines
(https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
https://www.embopress.org/page/journal/14602075/authorguide#expandedview

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

Further information is available in our Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (8th Jan 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

To the editor:

In their article "Scalable RT-LAMP-based SARS-CoV-2 testing for infection surveillance with applications in pandemic control", Lou et al. describe in great detail an RT-LAMP-based SARS-CoV-2 testing setup established in a purely academic setting during the ongoing pandemic, using previously published methods.

I greatly appreciate publishing a method at the given level of detail, comprising all SOPs, software code, validation data, and practical experience. However, the composition of this piece is uncommon even for a resource paper due to a lack of scientific conclusions; instead, this work reads in parts as a review article, adding valuable but often anecdotal experience.

Results:
• The results in Fig. 1 are interesting, albeit fall short of allowing to draw many convincing conclusions: First, in Fig. 1B the spread of taking two samples without interventions like eating of toothbrushing is missing in order to assess the spread originating from repeated human sampling; both interventions should be compared to the distribution without an intervention to conclude a statistically significant influence of interventions. In the text on p. 5, the statement "this effect was less prominent" is not justified because even with the current statistical model there is no statistically significant effect.
• The observation that centrifugation lowers viral genome concentration in the supernatant is interesting and convincing but not surprising; it is not clear how this observation is related to the overall method.
• Fig. 1D has to be performed with RT-qPCR as a quantitative readout, since RT-LAMP is absolutely not quantitative as shown in Fig. 3A, where the standard deviation is around 4 Ct values as gauged by eye. The relevant conclusions from Fig. 1D are not related to RT-LAMP, but rather to RNA concentration, so need to be quantitatively validated.
• Fig S2D: The linear correlation is not the critical bar, but rather the unity of the two methods' results; therefore, the black line should be f(x)=x, or the authors should quantify a relative loss for one over the other method. Why are there not the same number of dots as in S2C? Why is there no strong outlier for the "Ct 30" sample, which had very heterogenous curves in S2C? C and D should show the same experiment, as the curves alone are of low value without corresponding Ct values.
• In Fig. 2C, bottom right panel, one spike-in control seems to be dropping out; is this indicating a dropout rate of 1/96, around 1%? This should be discussed as it would be critically high. Maybe it's just the negative control well is plotted with the wrong color.
• Fig. 5B: The authors need to discuss or follow up on how two LAMP-negative samples could lead to around ten thousand unique virus-matching reads, which seems technically impossible.

Discussion:
The discussion is of low information content and could be collapsed to just the last paragraph.
• "due to storage space limitations and the regulations governing the storage of clinical samples, we had to dispose most samples" - anecdotal and not so interesting.
• p 24: "intrinsic limitations of simple RT-LAMP assays using colorimetric readouts without sequence-specific probes such as

molecular beacons, complicate its practical implementation." - but what's the authors' solution that solves this?
• p 24: "Using self-produced Bst-LF polymerase and HIV-RT preparations we found that slight modifications of the sample analysis procedures" - this would be valuable information, but details are missing.
• p 24: "efficient and cost-effective protocol" - the authors need to substantiate this important claim with a time- and cost calculation of RT-LAMP vs. RT-qPCR. Actual differences might be large, but might also be unexpectedly minor considering the expensive plate reader, 384-well cycler, commercial enzyme mix, number of steps. If large advantages in efficiency or cost are substantiated, this important information needs to be available to the reader.
• p 25: "In surveillance testing, even very low false-positive rates of a diagnostic test can be a problem as they can significantly affect the measured incidence" - the authors might want to avoid statements like this, as for the individual it is rather a problem to be confined to unjustified quarantine; please revisit also the statement "In that case, a low false-positive rate does not matter."
• p 25: "RT-qPCR testing offered by commercial providers usually reports back results after 24 - 72 hours, thus making such testing almost useless for surveillance testing. Our results were available approx. 5-6 hours ", this seems to be an unjustified statement. RT-qPCR could be performed in largely the same timeframe. Many commercial labs are set up to report RT-qPCR results within a few hours.

Minor points:
• p 5: averaged Δ(Ct[after]-Ct[before]) = -2.07, this should be a positive value if [after] has higher Ct than [before], please check also other formulas
• p 29: "[DaoThi'20, Herbst'21]" replace by a proper citation
• Fig 2D: "LAMP inc." is not explained
• p 13: "Ct 34 and larger" should be "Ct 34 and smaller" or "and higher viral loads"
• p 15: "anonymized" should be "pseudonymized", because access codes can be unblinded by authorized personnel
• It seems a lot of manual work to take the LAMP plates from the cycler to the plate reader and back every 5 minutes if only the 20-minutes timepoint is used by the software. The authors hint at manual inspection of time courses in some cases. If that's part of the workflow, it needs to be covered in detail.
• p 24: "Roehr et al." - citing own manuscript "in preparation" without naming a scientific result should be avoided.
• Very important for the throughput of this method is the barcoded tubes. These should be discussed in more detail: Type of barcode and ID, human readability, guaranteed uniqueness, type of cap, efficient de-capping process, mass-availability of tubes should be clearer.

Referee #2:

Summary
In this article, the authors described their implementation of high-throughput, RT-LAMP based SARS-CoV-2 surveillance in a university campus setting. Given the need for continuous surveillance of a range of pathogens, approaches for large-scale systems need to be established. This study could therefore be an important contribution for this endeavor. The method is well described, and could likely be well reproduced, with the exception of the analysis/visualization part, which I could not reproduce based on the information in the code repository. Overall, the study is well written, and could well be published with revisions as detailed below.

Major issues
• Introduction: the availability of PCR machines might indeed be be a topic, however personnel and consumables (there was e.g. a shortage of pipet tips and nucleic acids at that time) are also worth mentioning. Ideally, the authors should state to which extent their setup can provide relief in these aspects.
• The introduction should be broadened a bit in order to put RT-LAMP in a better context, by citing e.g. pre-SARS-CoV-2 RT-LAMP (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6425734/) or e.g. https://www.sciencedirect.com/science/article/pii/B9780128191781000484. Also, for comparison to RT-qPCR it would be interesting to take https://www.medrxiv.org/content/10.1101/2021.01.19.21250079v1 and/or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9011764/ into account. For the discussion around the usefulness, comparison with studies such as https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246544 or https://www.sciencedirect.com/science/article/pii/S2666776221000636?via%3Dihub should be included.
• Please review all figure legends in detail whether all figure elements are described in the legend or the figure itself (e.g the red vs blue dots in Fig. 5A).
• The github data repository is good, however add example files and clear instructions how to run the scripts using these example files.
• In the discussion, the authors write that their approach "can likely be maintained in many different academic institutions". It would however also be interesting to discuss how such an approach could be established in more general settings.

Minor issues
• Abstract, first line: rather "capacities".
• Second sentence: please specify "alternative" - cheaper, higher throughput, less investments into devices needed...
• Figure 1: the sentence in the legend for D, "Colors and shapes indicate individual patients" should also be mentioned for B.

Also, what is the dashed line? Figure 1D is a bit confusing, consider altering the drawing of time line on top a bit and better labeling of figure axis.
• Page 9, what does "SOP1"/"SOP2" refer to?
• Fig. 2C, the colored lines are difficult to see. Consider making them stronger, put them on top of the bundle of brown lines, and make colors more distinguishable.
• Fig. S2D, add the slope value. Also, the panels of supplementary figure 2 are not addressed in the text.
• Page 9: the positive controls are not clearly explained. Are the positive controls in Fig. 2 the Zika RNA ones?
• "which was higher than what could have been expected for unbiased surveillance testing" - one could also say that an unbiased testing obviously leads to higher values. It is interesting to compare the number with incidences, but this part of the sentence is rather to be omitted.
• Variant sequencing: instead of Fig. 5C, rather show which variant was detected at which time point.
• The symbol for heat inactivation in Fig. 4B is a bit misleading given the information in the methods.

Point-by-Point response

Referee #1:

To the editor:

In their article "Scalable RT-LAMP-based SARS-CoV-2 testing for infection surveillance with applications in pandemic control", Lou et al. describe in great detail an RT-LAMP-based SARS-CoV-2 testing setup established in a purely academic setting during the ongoing pandemic, using previously published methods.

I greatly appreciate publishing a method at the given level of detail, comprising all SOPs, software code, validation data, and practical experience. However, the composition of this piece is uncommon even for a resource paper due to a lack of scientific conclusions; instead, this work reads in parts as a review article, adding valuable but often anecdotal experience.

Results:
- The results in Fig. 1 are interesting, albeit fall short of allowing to draw many convincing conclusions: First, in Fig. 1B the spread of taking two samples without interventions like eating of toothbrushing is missing in order to assess the spread originating from repeated human sampling; both interventions should be compared to the distribution without an intervention to conclude a statistically significant influence of interventions.
Re: Thanks for your suggestions. We have assessed the background fluctuation of the viral load between two samples with a sampling interval between 5-30 minutes. The results were added in the revised manuscript on p.5: "*We evaluated the impacts of specific behavior of the test subjects or sample handling on SARS-CoV-2 diagnosis by RT-qPCR (Figure 1B-D). Without any interventions in a 5-30 minutes sampling interval, the average ΔCt of repeated sampling is -0.22 (95% confidence interval (CI) [-1.19, 0.75], p-value = 0.633) (Figure S1B). In contrast, food intake resulted in an increase of the Ct values by approximately two cycles"* (Changes in magenta). We also revised the Figure S1B on p.8 to show the Ct value fluctuation of repeated samples.

- In the text on p. 5, the statement "this effect was less prominent" is not justified because even with the current statistical model there is no statistically significant effect.
Re: In the revised manuscript we changed this statement to (p.5): *"For tooth brushing (sample taken earliest 10 min after teeth brushing) no significant reduction in the viral load could be observed (Δ = -1.04; 95% CI [-2.32, 0.25]; p-value = 0.11)."* (Changes in magenta).

- The observation that centrifugation lowers viral genome concentration in the supernatant is interesting and convincing but not surprising; it is not clear how this observation is related to the overall method.
Re: Centrifugation of the gargle samples is an important step in the sample handling workflow to allow clean de-capping of the sample tubes to avoid contamination. It thus was important to know how centrifugation influences the viral load. Based on this result we reduced centrifugation to the absolutely necessary minimum. To make this clear in the revised manuscript, the following sentence was added on p.5: *"Furthermore, we observed that centrifugation of the sample (3500g for 1 min) increased the Ct values on average by almost two (Δ = -1.76; 95% CI [-2.45, -1.08]; p-value < 0.0001), indicating that a large amount of the virus is present in larger particles or infected cells that can be easily*

sedimented (Figure 1C). Therefore, we kept centrifugation of gargle samples minimal in our workflow." (Changes in magenta).

- Fig. 1D has to be performed with RT-qPCR as a quantitative readout since RT-LAMP is absolutely not quantitative as shown in Fig. 3A, where the standard deviation is around 4 Ct values as gauged by eye. The relevant conclusions from Fig. 1D are not related to RT-LAMP, but rather to RNA concentration, so need to be quantitatively validated.

Re: Thanks for your suggestion. Unfortunately, at the time point when we did the experiments, we did not have the necessary PCR capacity to run these Experiments by qPCR. Meanwhile the samples are destroyed. However, the important matter here is the fact that the qualitative results, whether a sample is positive or negative is not affected by inactivation or storage of the sample. To emphasizes that this is not a quantitative result that refers to exact influences on virus load, we introduced a small change on p.6: *"We observed that the heat inactivation step and subsequent storage of the samples at 4°C (for later validation of positive RT-LAMP results) did not negatively influence the detection of the virus (Figure 1D (ii; iii)). Together these experiments suggested a convenient and robust sample collection and handling protocol for SARS-CoV-2 diagnostics."* (Changes in magenta).

- Fig S2D: The linear correlation is not the critical bar, but rather the unity of the two methods' results; therefore, the black line should be f(x)=x, or the authors should quantify a relative loss for one over the other method. Why are there not the same number of dots as in S2C? Why is there no strong outlier for the "Ct 30" sample, which had very heterogenous curves in S2C? C and D should show the same experiment, as the curves alone are of low value without corresponding Ct values.

Re: Fig. S2C (Fig. EV2C in the revised version) is showing the comparison of automated and manual RNA isolation using a serial dilution of one positive sample. Due to the heterogeneity of gargle samples, no perfect match of the results can be expected. In Fig. S2D (Fig. EV2D in the revised version), we further evaluate automated RNA isolation using different positive samples with low viral loads. In the revised manuscript, we added the equation of linear regression to the Fig. S2D (Fig. EV2D in the revised version), and also revised the text on p.9: *"To accommodate high numbers of samples, we implemented and validated an automated RNA extraction with a laboratory liquid handling device (Hamilton NGS STAR), which resulted in a time saving of ~55 min (40%) for one sample plate at similar quality (Supplementary Figure EV2A-EV2B, SOP1). To compare the efficiency of the automated RNA isolation protocol with the manual RNA isolation protocol, we applied both protocols using a 3-fold serial dilution series of one SARS-CoV-2 gargle sample (Ct = 30. Viral RNA could be detected by RT-qPCR in samples diluted up to 81-fold after either automated or manual RNA isolation. Either of the two protocols showed amplification curves with an interval of 1.5 Ct values for each 3-fold dilution step (Supplementary Figure EV2C). We also evaluated the automated RNA isolation protocol using several different weakly positive samples (Ct > 31), using automated and manual RNA isolation in parallel. The Ct values of isolated RNA from both protocols were in good agreement (Figure EV2D), indicating that automated RNA isolation is robust."* (Changes in magenta).

- In Fig. 2C, bottom right panel, one spike-in control seems to be dropping out; is this indicating a dropout rate of 1/96, around 1%? This should be discussed as it would be critically high. Maybe it's just the negative control well is plotted with the wrong color.
Re: Thanks for pointing this out. Fig 2C is meant to include all the possible scenarios that could happen but not necessarily happen frequently in our routine testing. The dropping out of spike-in control is usually caused by either a robot pipette error or a difficult gargle sample containing substances interfering with the extraction reagents.

- Fig. 5B: The authors need to discuss or follow up on how two LAMP-negative samples could lead to around ten thousand unique virus-matching reads, which seems technically impossible.
Re: In the text we describe that these two samples were false negative by RT-PCR. By RT-LAMP and LAMP-sequencing they were in fact positive. Here the corresponding text on p.20: "*In contrast, two RT-LAMP positive samples that did not yield a positive RT-qPCR result turned out to be positive by RT-LAMP-sequencing. These results indicate a low false-positive rate in our surveillance testing strategy and also demonstrate that there are intrinsic technical limitations for both methods RT-qPCR and RT-LAMP when it comes to the exact diagnosis of samples with extremely weak viral load*."

Discussion:
The discussion is of low information content and could be collapsed to just the last paragraph.
Re: In this revised manuscript, irrelevant information was removed from the discussion section and some statements were rephrased. Since the discussion part is largely restructured, please refer to p.25 - p26 for the detailed changes.

• "due to storage space limitations and the regulations governing the storage of clinical samples, we had to dispose most samples" - anecdotal and not so interesting.
Re: This statement has been deleted.

• p 24: "intrinsic limitations of simple RT-LAMP assays using colorimetric readouts without sequence-specific probes such as molecular beacons, complicate its practical implementation." - but what's the authors' solution that solves this?
Re: This statement has been deleted as it is not relevant to our main focus.

• p 24: "Using self-produced Bst-LF polymerase and HIV-RT preparations we found that slight modifications of the sample analysis procedures" - this would be valuable information, but details are missing.
Re: This is beyond the scope of this paper, as we did not use the self-produced enzymes for the tests shown. However, we felt it was important to provide a reference and an indication that this is indeed possible.

• p 24: "efficient and cost-effective protocol" - the authors need to substantiate this important claim with a time- and cost calculation of RT-LAMP vs. RT-qPCR. Actual differences might be large, but might also be unexpectedly minor considering the expensive plate reader, 384-

well cycler, commercial enzyme mix, number of steps. If large advantages in efficiency or cost are substantiated, this important information needs to be available to the reader.
Re: In this revised manuscript, we described in detail the start-up and implementation costs of RT-LAMP test station on p.18 – p.19. We also discussed about the time and cost comparison between RT-LAMP and RT-qPCR on p.25- p.26.

• p 25: "In surveillance testing, even very low false-positive rates of a diagnostic test can be a problem as they can significantly affect the measured incidence" - the authors might want to avoid statements like this, as for the individual it is rather a problem to be confined to unjustified quarantine; please revisit also the statement "In that case, a low false-positive rate does not matter."
Re: The perception of false positive results changed dramatically during the pandemic. In the early phase it was important to catch them all to stop transmission chains and unjustified quarantine due to a false positive result was not seen as a problem for the individual, but rather a problem that caused a lot of work in contact tracing. Later, things changed obviously.

• p 25: "RT-qPCR testing offered by commercial providers usually reports back results after 24 - 72 hours, thus making such testing almost useless for surveillance testing. Our results were available approx. 5-6 hours ", this seems to be an unjustified statement. RT-qPCR could be performed in largely the same timeframe. Many commercial labs are set up to report RT-qPCR results within a few hours.
Re: Thanks for your suggestions. We rephrased some statements in the discussion and also added the discussion about time and cost comparison between RT-LAMP and RT-qPCR.

Minor points:
- p 5: averaged Δ(Ct[after]-Ct[before]) = -2.07, this should be a positive value if [after] has higher Ct than [before], please check also other formulas
Re: We apologize for this mistake. The formula was given wrong. This has been corrected accordingly in the revised manuscript on p.5: *"This revealed an increase of the Ct values by approximately two cycles for food intake (averaged Δ(Ct[before]-Ct[after]) = -2.07; 95% confidence interval (CI) [-4.02, -0.13]; p-value = 0.04)."* (Changes in magenta).

- p 29: "[DaoThi'20, Herbst'21]" replace by a proper citation.
Re: The citation was corrected on p. 29: *"LAMP-sequencing was performed and analyzed as previously described* (Dao Thi *et al.*, 2020; Herbst *et al.*, 2021) *except using primer P7nxt-N2-Lbrc … NGS library preparation to accommodate that the RT-LAMP reactions were performed with the N2 primer set."* (Changes in magenta).

- Fig 2D: "LAMP inc." is not explained
Re: We revised the figure legend accordingly on p. 11/ Fig. 2D: *"(D) Annotation of the decision matrix. (+) indicates ΔOD ≧ 0.1 at 20 min; (-) indicates ΔOD < 0.1 at 20 min. LAMP positive (3/3 positive replicates) and LAMP weakly positive/LAMP inc. (1/3 or 2/3 positive replicates) results were validated by RT-qPCR."* (Changes in magenta).

- p 13: "Ct 34 and larger" should be "Ct 34 and smaller" or "and higher viral loads"
Re: The phrasing was altered accordingly on p. 13: *"Taken together, in our implementation of automated SARS-CoV-2 testing all samples with a diagnostic Ct value of Ct34 and higher viral loads contained at least one replicate that scored positive in the RT-LAMP assay (285*

of 285), whereas 83.3% of the samples with Ct values between 34 and 36 were tested positive with at least one replicate in the RT-LAMP assay (20 of 24).” (Changes in magenta).

- p 15: "anonymized" should be "pseudonymized", because access codes can be unblinded by authorized personnel
Re: We changed the wording accordingly on p. 15: *“All samples were identified only by barcode and access code, i.e., pseudonymized.”* (Changes in magenta).

- It seems a lot of manual work to take the LAMP plates from the cycler to the plate reader and back every 5 minutes if only the 20-minutes timepoint is used by the software. The authors hint at manual inspection of time courses in some cases. If that's part of the workflow, it needs to be covered in detail.
Re: We added the following sentence on p. 10: “*The test station personnel verified the assignments by the tool and manually inspected the time course measurements of samples with unusual or borderline appearances to adjust the automatic assignments if necessary. The diagnostic results were then directly uploaded to a database, where test participants could retrieve their individual results via a webpage.”* (Changes in magenta).

- p 24: "Roehr et al." - citing own manuscript "in preparation" without naming a scientific result should be avoided.
Re: The paper has been published and is now fully cited.

- Very important for the throughput of this method is the barcoded tubes. These should be discussed in more detail: Type of barcode and ID, human readability, guaranteed uniqueness, type of cap, efficient de-capping process, mass-availability of tubes should be clearer.
Re: We described more details about the Micronic tubes on p.5: *“The pointy tip of the bottle is used to dispense ~1 ml of the gargling fluid into a 1.4ml Micronic tube (Micronic, Lelysta, Netherlands), which is returned to the test station for analysis (Figure 1A). The Micronic tubes are produced with thread barcode surface that can never wear or fall off and can be easily scanned to keep good track of the samples. With the screw cap, the Micronic tube can be rapidly decapped with a multi-channel decapper. In addition, the tubes can be processed directly by standard liquid handling platforms, thus avoiding the need for manual pipetting steps in the laboratory and thereby decreasing human error as well as infection and contamination risks.”* (Changes in magenta).

Referee #2:

Summary
In this article, the authors described their implementation of high-throughput, RT-LAMP based SARS-CoV-2 surveillance in a university campus setting. Given the need for continuous surveillance of a range of pathogens, approaches for large-scale systems need to be established. This study could therefore be an important contribution for this endeavor. The method is well described, and could likely be well reproduced, with the exception of the analysis/visualization part, which I could not reproduce based on the information in the code repository. Overall, the study is well written, and could well be published with revisions as detailed below.

Major issues

- Introduction: the availability of PCR machines might indeed be be a topic, however personnel and consumables (there was e.g. a shortage of pipet tips and nucleic acids at that time) are also worth mentioning. Ideally, the authors should state to which extent their setup can provide relief in these aspects.
Re: Thanks for your suggestion. Since consumables and personnel with relevant knowledge and skills are required for both PCR and LAMP assays, so we didn't talk about that in the introduction. However, in the revised manuscript, we reported the detailed running costs of our RT-LAMP test station in the results section, showing the cost-efficiency of our RT-LAMP assay.

- The introduction should be broadened a bit in order to put RT-LAMP in a better context, by citing e.g. pre-SARS-CoV-2 RT-LAMP (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6425734/) or e.g. https://www.sciencedirect.com/science/article/pii/B9780128191781000484. Also, for comparison to RT-qPCR it would be interesting to take https://www.medrxiv.org/content/10.1101/2021.01.19.21250079v1 and/or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9011764/ into account. For the discussion around the usefulness, comparison with studies such as https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246544 or https://www.sciencedirect.com/science/article/pii/S2666776221000636?via%3Dihub should be included.
Re: Thanks for your suggestion. We have revised the introduction accordingly on p.3 - p.4: *"Very early in the pandemic loop-mediated isothermal amplification (LAMP)* (Nagamine *et al*, 2002; Notomi *et al*, 2000) *coupled with a reverse transcriptase (RT-LAMP) was developed and validated as an alternative viral genome detection method* (Alekseenko *et al*, 2021; Zhang *et al*, 2020a) . *With high sensitivity and specificity, RT-LAMP is an easy to implement, rapid and cost-effective alternative to RT-qPCR tests* (Kellner *et al*, 2021; Kellner *et al*, 2022). *RT-LAMP is a particularly attractive substitute for RT-qPCR because it does not depend on the availability of specialized equipment such as thermocyclers. A variety of isothermal instruments heated from various energy sources could be used in RT-LAMP assay* (Snodgrass *et al*, 2018). *... We finished validation of the method within a short period (Dao Thi et al., 2020; Herbst et al., 2021; Klein et al, 2020; Ludwig et al., 2021) and started using the colorimetric RT-LAMP as an alternative to commercial RT-qPCR testing capacity for sentinel studies and surveillance testing of larger groups of people. Examination of representative samples of a certain population is very important to assess the prevalence of SARS-CoV-2 in the whole population* (Willeit *et al*, 2021)*."* (Changes in magenta).

- Please review all figure legends in detail whether all figure elements are described in the legend or the figure itself (e.g the red vs blue dots in Fig. 5A).
Re: The legends of all figures have been corrected accordingly in the revised manuscript.

- The github data repository is good, however add example files and clear instructions how to run the scripts using these example files.
Re: The GitHub data repository was updated with example files and instructions (README file) accordingly.

- In the discussion, the authors write that their approach "can likely be maintained in many different academic institutions". It would however also be interesting to discuss how such an approach could be established in more general settings.

Re: Implementation is possible everywhere there is the relevant knowledge available. To emphasize the need of the trained stuff we extended our statement on p.26: *"Routine operation of such an RT-LAMP test station with student assistants is comparatively inexpensive and can likely be maintained in many different academic institutions and even more general settings, as long as knowledgeable staff is available, to provide scalable test capacity to increase the number of tests, e.g., if a future SARS-CoV-2 mutant or other pathogens require it."* (Changes in magenta).

Minor issues

- Abstract, first line: rather "capacities".

Re: We corrected the word accordingly on p.1: *"limited diagnostic capacities prevented sentinel testing"* (Changes in magenta).

- Second sentence: please specify "alternative" - cheaper, higher throughput, less investments into devices needed…

Re: We revised the sentence accordingly on p.1: *"Here, we describe the set-up of a cost-effective platform in a high-throughput manner,"* (Changes in magenta).

- Figure 1: the sentence in the legend for D, "Colors and shapes indicate individual patients" should also be mentioned for B. Also, what is the dashed line? Figure 1D is a bit confusing, consider altering the drawing of time line on top a bit and better labeling of figure axis.

Re: We revised the legend for Fig 1B and Fig 1C accordingly on p.8. In Fig 1B, dots on the dashed line indicates that the Ct values of samples collected before and after eating/toothbrushing are the same.

- Page 9, what does "SOP1"/"SOP2" refer to?

Re: Sorry for the confusion. SOP means 'Standard operation procedure', both of which are now referenced properly: *see also the corresponding Standard Operation Procedure which is provided as Supplementary File SOP1* resp. *SOP2* (p.9. and p.25.)

- Fig. 2C, the colored lines are difficult to see. Consider making them stronger, put them on top of the bundle of brown lines, and make colors more distinguishable.

Re: Fig 2C is showing the differences in absorbance (ΔOD) of each well at 434nm and 560nm during 30-min incubation of the RT-LAMP assay. Here we use the same color code with Fig 2A and 2B to be consistent. Since the positive samples (brown) have similar viral load with the positive controls (green), the curves are overlapping and not clearly distinguishable. While this is not easy to be seen in this figure that shows a screenshot from our user interface, in the user interface itself (Fig EV3), each amplification curve can be highlighted individually by simply clicking on it, in order to distinguish one sample from the other samples and controls. To explain this, we now introduced one additional sentence into the legend to Fig 2C on p.11: *"(C) Time-course analysis of the color change (ΔOD) of the LAMP reaction. Controls as indicated in (A). The plots represent screenshots from our user interface. In the user interface itself (Fig S3), each amplification curve can be highlighted individually by simply clicking on it, in order to distinguish it from the other samples and controls. "* (Changes in magenta).

- Fig. S2D, add the slope value. Also, the panels of supplementary figure 2 are not addressed in the text.
Re: In the revised manuscript, we added the equation of linear regression to the Fig. S2D (Fig. EV2D in this revised version), and also addressed the figure on p.9: *"To accommodate high numbers of samples, we implemented and validated an automated RNA extraction with a laboratory liquid handling device (Hamilton NGS STAR), which resulted in a time saving of ~55 min (40%) for one sample plate at similar quality (Supplementary Figure EV2A-EV2B, SOP1). To compare the efficiency of the automated RNA isolation protocol with the manual RNA isolation protocol, we applied both protocols using a 3-fold serial dilution series of one SARS-CoV-2 gargle sample (Ct = 30. Viral RNA could be detected by RT-qPCR in samples diluted up to 81-fold after either automated or manual RNA isolation. Either of the two protocols showed amplification curves with an interval of 1.5 Ct values for each 3-fold dilution step (Supplementary Figure EV2C). We also evaluated the automated RNA isolation protocol using several different weakly positive samples (Ct > 31), using automated and manual RNA isolation in parallel. The Ct values of isolated RNA from both protocols were in good agreement (Figure EV2D), indicating that automated RNA isolation is robust."* (Changes in magenta).

- Page 9: the positive controls are not clearly explained. Are the positive controls in Fig. 2 the Zika RNA ones?
Re: Sorry for the confusion. The positive controls are SARS-CoV-2 positive samples, whereas the Zika RNA is used as an internal control that is included in the lysis buffer during RNA isolation. In the revised manuscript, we modified the text on p. 9: *"Per 96-position rack, 92 Micronic tubes with test samples and four controls were included (Figure 2A). Three SARS-CoV-2 positive samples with known concentrations (with Ct value of 29, 32, and 36 respectively) were used as positive controls to evaluate the performance of RNA isolation and RT-LAMP assay. In addition, a negative control (saline) was incorporated to determine intra-assay contaminations."* (Changes in magenta).

- "which was higher than what could have been expected for unbiased surveillance testing" - one could also say that an unbiased testing obviously leads to higher values. It is interesting to compare the number with incidences, but this part of the sentence is rather to be omitted.
Re: Thanks for your kind suggestion. This sentence has been deleted in the revised manuscript on p.19.

• Variant sequencing: instead of Fig. 5C, rather show which variant was detected at which time point.
Re: Our study protocol approved by the ethics commission required that we anonymize the samples before sequencing, which made it impossible to track them back to individual dates.

- The symbol for heat inactivation in Fig. 4B is a bit misleading given the information in the methods.
Re: We're sorry for the confusion. In Fig 4B the symbol was meant to indicate the scanning of the sample. We now also included an additional symbol for heat inactivation.

Dear Michael,

Thank you for submitting your manuscript to EMBO Reports. I am sorry for the slow response. We have evaluated your responses to the referees and executed our QC process. We will be pleased to publish the paper as a 'resource' pending satisfactory minor revision as outlined below:

1) please see the attached editorial comments inserted in italics/green in your p-b-p response. Please review and address as appropriate;
2) Our screening process highlighted the following.
(i) Deleted Author: Dan Lou #5 >> please explain why the author was deleted and provide evidence Dan Lou agrees with that authorship change. Please also state that all authors agree to the additional authors added to the paper.
(ii) Please suggest up to 5 keywords that describe your paper.
(iii) please enhance the "Data availability section (see https://www.embopress.org/page/journal/14693178/authorguide#dataavailability):
Each dataset should include 1) a short description of the measurement type (eg RNA-Seq, ChIP-Seq, mass spectrometry proteomics, imaging, etc...), 2) the name of the repository (or its recommended acronym, below and consult fairsharing.org); 3) the DOI or accession number of the dataset; and 4) a resolvable link to the dataset, either in the form of a resolvable link from http://identifiers.org or as the full URL to the respective database record.
(iv) Disclosure and Competing Interests Statement:
We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Also, please rename the 'Conflict of Interests' section as 'Disclosure statement and competing interests'
(v) AC/CRediT: Should be removed from the ms. CRediT has replaced the traditional author contributions section because it offers a systematic machine readable author contributions format that allows for more effective research assessment. You are encouraged to use the free text boxes beneath each contributing author's name to add specific details on the author's contribution. More information is available in our guide to authors.
(vi) FUNDING INFO: this should be included in the Acknowledgement section.
(vii) Fig EV5A&B callouts are missing.
Remove all mention of "Supplementary/Supplemental".
(viii)
- please remove figures and tables from the manuscript file.
- The figure legends should follow after the References. Add headings 'Figure Legends' and 'Expanded View Figure Legends'.
- There are 2 files Supplementary SOP1 and SOP 2. This information should either be added to the main manuscript Materials and Methods section, or could be uploaded in one Appendix pdf file.

I would like to invite you to submit a revised version of the manuscript as soon as possible as the pandemic turns endemic, clearly the relevance of the study to this particular public health crisis is progressively reduced.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Best regards,

Bernd

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Point-by-Point response

Referee #1:

To the editor:

In their article "Scalable RT-LAMP-based SARS-CoV-2 testing for infection surveillance with applications in pandemic control", Lou et al. describe in great detail an RT-LAMP-based SARS-CoV-2 testing setup established in a purely academic setting during the ongoing pandemic, using previously published methods.

I greatly appreciate publishing a method at the given level of detail, comprising all SOPs, software code, validation data, and practical experience. However, the composition of this piece is uncommon even for a resource paper due to a lack of scientific conclusions; instead, this work reads in parts as a review article, adding valuable but often anecdotal experience.

Results:
- The results in Fig. 1 are interesting, albeit fall short of allowing to draw many convincing conclusions: First, in Fig. 1B the spread of taking two samples without interventions like eating of toothbrushing is missing in order to assess the spread originating from repeated human sampling; both interventions should be compared to the distribution without an intervention to conclude a statistically significant influence of interventions.
Re: Thanks for your suggestions. We have assessed the background fluctuation of the viral load between two samples with a sampling interval between 5-30 minutes. The results were added in the revised manuscript on p.5: "*We evaluated the impacts of specific behavior of the test subjects or sample handling on SARS-CoV-2 diagnosis by RT-qPCR (Figure 1B-D). Without any interventions in a 5-30 minutes sampling interval, the average ΔCt of repeated sampling is -0.22 (95% confidence interval (CI) [-1.19, 0.75], p-value = 0.633) (Figure S1B). In contrast, food intake resulted in an increase of the Ct values by approximately two cycles"* (Changes in magenta). We also revised the Figure S1B on p.8 to show the Ct value fluctuation of repeated samples.

>> BP: new data. Q: data on toothbrush intervention?
Several voluntary test subjects were asked to collect samples during their infections, and the exact time points of sample collection and behaviors such as eating, toothbrushing were recorded. Among these longitudinal samples, some of them were collected with a sampling interval between 5-30 minutes, without any intervention in between. Thus in this revision, we compared the CT values of these samples and observed that the background fluctuation of the viral load without any intervention is not statistically obvious. The source data is provided as "source data_figure EV1B".

- In the text on p. 5, the statement "this effect was less prominent" is not justified because even with the current statistical model there is no statistically significant effect.
Re: In the revised manuscript we changed this statement to (p.5): *"For tooth brushing (sample taken earliest 10 min after teeth brushing) no significant reduction in the viral load could be observed (Δ = -1.04; 95% CI [-2.32, 0.25]; p-value = 0.11)."* (Changes in magenta).

>> BP: OK

- The observation that centrifugation lowers viral genome concentration in the supernatant is interesting and convincing but not surprising; it is not clear how this observation is related to the overall method.

Re: Centrifugation of the gargle samples is an important step in the sample handling workflow to allow clean de-capping of the sample tubes to avoid contamination. It thus was important to know how centrifugation influences the viral load. Based on this result we reduced centrifugation to the absolutely necessary minimum. To make this clear in the revised manuscript, the following sentence was added on p.5: *"Furthermore, we observed that centrifugation of the sample (3500g for 1 min) increased the Ct values on average by almost two (Δ = -1.76; 95% CI [-2.45, -1.08]; p-value < 0.0001), indicating that a large amount of the virus is present in larger particles or infected cells that can be easily sedimented (Figure 1C). Therefore, we kept centrifugation of gargle samples minimal in our workflow."* (Changes in magenta).

>> BP: OK

- Fig. 1D has to be performed with RT-qPCR as a quantitative readout since RT-LAMP is absolutely not quantitative as shown in Fig. 3A, where the standard deviation is around 4 Ct values as gauged by eye. The relevant conclusions from Fig. 1D are not related to RT-LAMP, but rather to RNA concentration, so need to be quantitatively validated.

Re: Thanks for your suggestion. Unfortunately, at the time point when we did the experiments, we did not have the necessary PCR capacity to run these Experiments by qPCR. Meanwhile the samples are destroyed. However, the important matter here is the fact that the qualitative results, whether a sample is positive or negative is not affected by inactivation or storage of the sample. To emphasizes that this is not a quantitative result that refers to exact influences on virus load, we introduced a small change on p.6: *"We observed that the heat inactivation step and subsequent storage of the samples at 4°C (for later validation of positive RT-LAMP results) did not negatively influence the detection of the virus (Figure 1D (ii; iii)). Together these experiments suggested a convenient and robust sample collection and handling protocol for SARS-CoV-2 diagnostics."* (Changes in magenta).

>> BP: OK, but add a clearer not that RT-LAMP is non quantitative, but that this is not necessary in this qualitative assay. qPCR could be employed to quantify effects in more detail.

RT-LAMP can be quite quantitively but our implementation only provides a resolution of 5 min, which allows the discrimination between weak, medium and high viral load. But since we only use positive samples in the upper Ct range (with lower to low viral loads), to check for test sensitivity, we decided to stick to the three possible outcomes described in the decision tree shown in the Figure 2D.

- Fig S2D: The linear correlation is not the critical bar, but rather the unity of the two methods' results; therefore, the black line should be f(x)=x, or the authors should quantify a relative loss for one over the other method. Why are there not the same number of dots as in S2C? Why is there no strong outlier for the "Ct 30" sample, which had very heterogenous curves in S2C? C and D should show the same experiment, as the curves alone are of low value without corresponding Ct values.

Re: Fig. S2C (Fig. EV2C in the revised version) is showing the comparison of automated and manual RNA isolation using a serial dilution of one positive sample. Due to the heterogeneity of gargle samples, no perfect match of the results can be expected. In Fig. S2D (Fig. EV2D in the revised version), we further evaluate automated RNA isolation using different positive samples with low viral loads. In the revised manuscript, we added the equation of linear regression to the Fig. S2D (Fig. EV2D in the revised version), and also revised the text on p.9: *"To accommodate high numbers of samples, we implemented and validated an automated RNA extraction with a laboratory liquid handling device (Hamilton NGS STAR), which resulted in a time saving of ~55 min (40%) for one sample plate at similar quality (Supplementary Figure EV2A-EV2B, SOP1). To compare the efficiency of the automated RNA isolation protocol with the manual RNA isolation protocol, we applied both protocols using a 3-fold serial dilution series of one SARS-CoV-2 gargle sample (Ct = 30. Viral RNA could be detected by RT-qPCR in samples diluted up to 81-fold after either automated or manual RNA isolation. Either of the two protocols showed amplification curves with an interval of 1.5 Ct values for each 3-fold dilution step (Supplementary Figure EV2C). We also evaluated the automated RNA isolation protocol using several different weakly positive samples (Ct > 31), using automated and manual RNA isolation in parallel. The Ct values of isolated RNA from both protocols were in good agreement (Figure EV2D), indicating that automated RNA isolation is robust."* (Changes in magenta).

>> BP: the referee suggested 'the authors should quantify a relative loss for one over the other method': is this not feasible? 'good agreement' is a vague term. Can this point be made in a more definitive manner? Cosmetic: I suggest to change 'is robust' to 'can be applied without nagtive impact in the protocol'

We introduced the suggested change.

- In Fig. 2C, bottom right panel, one spike-in control seems to be dropping out; is this indicating a dropout rate of 1/96, around 1%? This should be discussed as it would be critically high. Maybe it's just the negative control well is plotted with the wrong color.

Re: Thanks for pointing this out. Fig 2C is meant to include all the possible scenarios that could happen but not necessarily happen frequently in our routine testing. The dropping out of spike-in control is usually caused by either a robot pipette error or a difficult gargle sample containing substances interfering with the extraction reagents.

>> BP: OK, but please add this to the discussion. Please address the frequency question: Is there a 1% failure rate?

All tests have a certain failure rate that might fluctuate from day to day and experiment to experiment etc. Please consider, this is an open platform, and not a CE certified enclosed system (which in some cases can have high failure rates due to erratic malfunctioning). Essentially, what this means is that one has to deal with the situation. As can be seen in the decision tree (Fig 2D), samples where the spike-in control fails are simply repeated. This is not "critical" but a routine situation that happens occasionally in diagnostics also with PCR

based testing etc. There are many possible reasons for this. If the problem persists for the same sample (which excludes a technical or handling error), participants were informed (using a feed-back comment function in the software) that the sample could not be analyzed and they are offered to repeat the test. This happened extremely rarely. We heard of one particular test participant (but never met in person) who produced samples that always failed in both our LAMP assay and also qPCR assay…our wild guessing suspected that cream for a denture or other exotic oral care products could be the reason …
To account for this, we now added a few lines in the revised manuscript on p.7: *"The test station personnel evaluated the RT-LAMP plates based on the decision tree and assigned a status for each sample assisted by the automated pre-classification of the "LAMP plate viewer tool". Unexpected results for the positive or the negative control samples, but correct spike-in control results were usually caused by errors in setting up the RT-LAMP assay, in which (rare) case we simply repeated the assay. If the spike-in control dropped out in a specific sample, we included this sample into the next round of analysis (Figure 2D). Such spike-in control failures were rare (failure rate 0.8%) and were usually caused by difficult gargle samples containing substances interfering with the RNA extraction procedure. In addition, the test station personnel also verified the assignments by the tool and manually inspected the time course measurements of samples with unusual or borderline appearances to adjust the automatic assignments if necessary."*

- Fig. 5B: The authors need to discuss or follow up on how two LAMP-negative samples could lead to around ten thousand unique virus-matching reads, which seems technically impossible.
Re: In the text we describe that these two samples were false negative by RT-PCR. By RT-LAMP and LAMP-sequencing they were in fact positive. Here the corresponding text on p.20: "*In contrast, two RT-LAMP positive samples that did not yield a positive RT-qPCR result turned out to be positive by RT-LAMP-sequencing. These results indicate a low false-positive rate in our surveillance testing strategy and also demonstrate that there are intrinsic technical limitations for both methods RT-qPCR and RT-LAMP when it comes to the exact diagnosis of samples with extremely weak viral load*."

BP: OK, but an the false +ve rate be estimated (both for qPCR and LAMP)?

No, the number of such cases observed were too small to do meaningful statistics on this.

Discussion:
The discussion is of low information content and could be collapsed to just the last paragraph.
Re: In this revised manuscript, irrelevant information was removed from the discussion section and some statements were rephrased. Since the discussion part is largely restructured, please refer to p.25 - p26 for the detailed changes.
>> BP: OK

• "due to storage space limitations and the regulations governing the storage of clinical samples, we had to dispose most samples" - anecdotal and not so interesting.

Re: This statement has been deleted.
>> BP: OK

• p 24: "intrinsic limitations of simple RT-LAMP assays using colorimetric readouts without sequence-specific probes such as molecular beacons, complicate its practical implementation." - but what's the authors' solution that solves this?
Re: This statement has been deleted as it is not relevant to our main focus.
>> BP: OK

• p 24: "Using self-produced Bst-LF polymerase and HIV-RT preparations we found that slight modifications of the sample analysis procedures" - this would be valuable information, but details are missing.
Re: This is beyond the scope of this paper, as we did not use the self-produced enzymes for the tests shown. However, we felt it was important to provide a reference and an indication that this is indeed possible.
>> BP: OK to keep. For avoidance of doubt could remind the reader these are not used in the data shown and details are not displayed as it depends on the individual preparation protocol applied.

During the restructuring of the discussion in the revised version (as requested), we removed one sentence so that it is clear that we did not use self-purified enzymes. We forgot to list this change here, sorry.

• p 24: "efficient and cost-effective protocol" - the authors need to substantiate this important claim with a time- and cost calculation of RT-LAMP vs. RT-qPCR. Actual differences might be large, but might also be unexpectedly minor considering the expensive plate reader, 384-well cycler, commercial enzyme mix, number of steps. If large advantages in efficiency or cost are substantiated, this important information needs to be available to the reader.
Re: In this revised manuscript, we described in detail the start-up and implementation costs of RT-LAMP test station on p.18 – p.19. We also discussed about the time and cost comparison between RT-LAMP and RT-qPCR on p.25- p.26.
>> BP: OK – thanks for adding. Is it apparent how much more would be saved by employing self-produced enzymes (as per above). Even if this is not the focus of the data here, it would be interesting for many users, especially in counties less well serve by suppliers/less well funded.
I agree and we should try to find time to write up this. In any case, we have all SOPs ready and are willing to send them out upon request. Since you mention this here, I added back the deleted sentence from the remark before and included a corresponding statement: "Using self-produced Bst-LF polymerase and HIV-RT preparations we found that slight modifications of the sample analysis procedures ensure similar sensitivity as compared to assays performed with the NEB enzyme mixtures (not included in the data shown here; SOPs available upon requests)."

• p 25: "In surveillance testing, even very low false-positive rates of a diagnostic test can be a problem as they can significantly affect the measured incidence" - the authors might want to avoid statements like this, as for the individual it is rather a problem to be confined to unjustified quarantine; please revisit also the statement "In that case, a low false-positive rate does not matter."
Re: The perception of false positive results changed dramatically during the pandemic. In the early phase it was important to catch them all to stop transmission chains and unjustified

quarantine due to a false positive result was not seen as a problem for the individual, but rather a problem that caused a lot of work in contact tracing. Later, things changed obviously.

BP: I think we'd all agree, but the point stands – also vs. a new pandemic. Please address texturally as suggested by the referee, also including your response on pandemic stage context/response policy. Note that these are not the same cf. China's 'zero COVID' policy.

Sorry, but we stick to our original statement, since we are talking about surveillance testing, and our statement is factually correct (e.g. see https://sph.umich.edu/news/2020posts/surveillance-testing-gathering-the-data-on-covid-19.html). To avoid false positive diagnosis, we validate all positives by RT-qPCR, as we describe in the subsequent text of this passage. The second statement "In that case, a low false-positive rate does not matter." has been deleted during the restructuring of the discussion in the revised manuscript (although in the strict sense of 'surveillance testing' it is also correct).

• p 25: "RT-qPCR testing offered by commercial providers usually reports back results after 24 - 72 hours, thus making such testing almost useless for surveillance testing. Our results were available approx. 5-6 hours ", this seems to be an unjustified statement. RT-qPCR could be performed in largely the same timeframe. Many commercial labs are set up to report RT-qPCR results within a few hours.
Re: Thanks for your suggestions. We rephrased some statements in the discussion and also added the discussion about time and cost comparison between RT-LAMP and RT-qPCR.

>> BP: we agree that a time saving point per se can only be made based on a side by side comparison. Please ensure this is not overclaimed anywhere in the article.
Agree, obviously PCR tests can be as speedy as RT-LAMP, which is the case for specific applications, e.g. testing at airports. But in the Heidelberg area I have not encountered a commercial lab that was faster than 24 hours turnaround time, and many that were much slower, so I simply specified the statement 'in the Heidelberg area', and I removed the statement ", thus making such testing almost useless for surveillance testing", which would require additional explanations.

Minor points:
- p 5: averaged Δ(Ct[after]-Ct[before]) = -2.07, this should be a positive value if [after] has higher Ct than [before], please check also other formulas
Re: We apologize for this mistake. The formula was given wrong. This has been corrected accordingly in the revised manuscript on p.5: "This revealed an increase of the Ct values by approximately two cycles for food intake (averaged Δ(Ct[before]-Ct[after]) = -2.07; 95% confidence interval (CI) [-4.02, -0.13]; p-value = 0.04)." (Changes in magenta).
>> BP: OK

- p 29: "[DaoThi'20, Herbst'21]" replace by a proper citation.
Re: The citation was corrected on p. 29: *"LAMP-sequencing was performed and analyzed as previously described* (Dao Thi *et al.*, 2020; Herbst *et al.*, 2021) *except using primer P7nxt-N2-Lbrc … NGS library preparation to accommodate that the RT-LAMP reactions were performed with the N2 primer set."* (Changes in magenta).
>> BP: OK

- Fig 2D: "LAMP inc." is not explained

Re: We revised the figure legend accordingly on p. 11/ Fig. 2D: *"(D) Annotation of the decision matrix. (+) indicates ΔOD ≧ 0.1 at 20 min; (-) indicates ΔOD < 0.1 at 20 min. LAMP positive (3/3 positive replicates) and LAMP weakly positive/LAMP inc. (1/3 or 2/3 positive replicates) results were validated by RT-qPCR."* (Changes in magenta).
>> BP: OK

- p 13: "Ct 34 and larger" should be "Ct 34 and smaller" or "and higher viral loads"
Re: The phrasing was altered accordingly on p. 13: *"Taken together, in our implementation of automated SARS-CoV-2 testing all samples with a diagnostic Ct value of Ct34 and higher viral loads contained at least one replicate that scored positive in the RT-LAMP assay (285 of 285), whereas 83.3% of the samples with Ct values between 34 and 36 were tested positive with at least one replicate in the RT-LAMP assay (20 of 24)."* (Changes in magenta).
>> BP: OK

- p 15: "anonymized" should be "pseudonymized", because access codes can be unblinded by authorized personnel
Re: We changed the wording accordingly on p. 15: *"All samples were identified only by barcode and access code, i.e., pseudonymized."* (Changes in magenta).
>> BP: OK

- It seems a lot of manual work to take the LAMP plates from the cycler to the plate reader and back every 5 minutes if only the 20-minutes timepoint is used by the software. The authors hint at manual inspection of time courses in some cases. If that's part of the workflow, it needs to be covered in detail.
Re: We added the following sentence on p. 10: "*The test station personnel verified the assignments by the tool and manually inspected the time course measurements of samples with unusual or borderline appearances to adjust the automatic assignments if necessary. The diagnostic results were then directly uploaded to a database, where test participants could retrieve their individual results via a webpage."* (Changes in magenta).
>> BP: OK

- p 24: "Roehr et al." - citing own manuscript "in preparation" without naming a scientific result should be avoided.
Re: The paper has been published and is now fully cited.
>> BP: OK

- Very important for the throughput of this method is the barcoded tubes. These should be discussed in more detail: Type of barcode and ID, human readability, guaranteed uniqueness, type of cap, efficient de-capping process, mass-availability of tubes should be clearer.
Re: We described more details about the Micronic tubes on p.5: *"The pointy tip of the bottle is used to dispense ~1 ml of the gargling fluid into a 1.4ml Micronic tube (Micronic, Lelysta, Netherlands), which is returned to the test station for analysis (Figure 1A). The Micronic tubes are produced with thread barcode surface that can never wear or fall off and can be easily scanned to keep good track of the samples. With the screw cap, the Micronic tube can be rapidly decapped with a multi-channel decapper. In addition, the tubes can be processed directly by standard liquid handling platforms, thus avoiding the need for manual pipetting steps in the laboratory and thereby decreasing human error as well as infection and contamination risks."* (Changes in magenta).
>> BP: OK

Referee #2:

Summary
In this article, the authors described their implementation of high-throughput, RT-LAMP based SARS-CoV-2 surveillance in a university campus setting. Given the need for continuous surveillance of a range of pathogens, approaches for large-scale systems need to be established. This study could therefore be an important contribution for this endeavor. The method is well described, and could likely be well reproduced, with the exception of the analysis/visualization part, which I could not reproduce based on the information in the code repository. Overall, the study is well written, and could well be published with revisions as detailed below.

Major issues
- Introduction: the availability of PCR machines might indeed be be a topic, however personnel and consumables (there was e.g. a shortage of pipet tips and nucleic acids at that time) are also worth mentioning. Ideally, the authors should state to which extent their setup can provide relief in these aspects.
Re: Thanks for your suggestion. Since consumables and personnel with relevant knowledge and skills are required for both PCR and LAMP assays, so we didn't talk about that in the introduction. However, in the revised manuscript, we reported the detailed running costs of our RT-LAMP test station in the results section, showing the cost-efficiency of our RT-LAMP assay.
>> BP: OK

- The introduction should be broadened a bit in order to put RT-LAMP in a better context, by citing e.g. pre-SARS-CoV-2 RT-LAMP (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6425734/) or e.g. https://www.sciencedirect.com/science/article/pii/B9780128191781000484. Also, for comparison to RT-qPCR it would be interesting to take https://www.medrxiv.org/content/10.1101/2021.01.19.21250079v1 and/or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9011764/ into account. For the discussion around the usefulness, comparison with studies such as https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246544 or https://www.sciencedirect.com/science/article/pii/S2666776221000636?via%3Dihub should be included.
Re: Thanks for your suggestion. We have revised the introduction accordingly on p.3 - p.4: *"Very early in the pandemic loop-mediated isothermal amplification (LAMP)* (Nagamine *et al*, 2002; Notomi *et al*, 2000) *coupled with a reverse transcriptase (RT-LAMP) was developed and validated as an alternative viral genome detection method* (Alekseenko *et al*, 2021; Zhang *et al*, 2020a). *With high sensitivity and specificity, RT-LAMP is an easy to implement, rapid and cost-effective alternative to RT-qPCR tests* (Kellner *et al*, 2021; Kellner *et al*, 2022). *RT-LAMP is a particularly attractive substitute for RT-qPCR because it does not depend on the availability of specialized equipment such as thermocyclers. A variety of isothermal instruments heated from various energy sources could be used in RT-LAMP assay* (Snodgrass *et al*, 2018). *... We finished validation of the method within a short period (Dao Thi et al., 2020; Herbst et al., 2021; Klein et al, 2020; Ludwig et al., 2021) and started using the colorimetric RT-LAMP as an alternative to commercial RT-qPCR testing capacity for sentinel studies and surveillance testing of larger groups of people. Examination of*

representative samples of a certain population is very important to assess the prevalence of SARS-CoV-2 in the whole population (Willeit *et al*, 2021)*."* (Changes in magenta).
>> BP: OK

- Please review all figure legends in detail whether all figure elements are described in the legend or the figure itself (e.g the red vs blue dots in Fig. 5A).
Re: The legends of all figures have been corrected accordingly in the revised manuscript.
>> BP: OK

- The github data repository is good, however add example files and clear instructions how to run the scripts using these example files.
Re: The GitHub data repository was updated with example files and instructions (README file) accordingly.
>> BP: OK

- In the discussion, the authors write that their approach "can likely be maintained in many different academic institutions". It would however also be interesting to discuss how such an approach could be established in more general settings.
Re: Implementation is possible everywhere there is the relevant knowledge available. To emphasize the need of the trained stuff we extended our statement on p.26: *"Routine operation of such an RT-LAMP test station with student assistants is comparatively inexpensive and can likely be maintained in many different academic institutions and even more general settings, as long as knowledgeable staff is available, to provide scalable test capacity to increase the number of tests, e.g., if a future SARS-CoV-2 mutant or other pathogens require it."* (Changes in magenta).

>> BP: Since application outside academic setting seems a key use case (cf. Africa), please add a short discussion re. skillset/training requirements esp. vs. PCR testing: is it easier/harder to learn? What does it take for existing diagnostic labs to apply?
We added the following text:
A royalty-free RT-LAMP based test station could also play an important role in African countries where the diagnostics industry is still underdeveloped and access to testing is expensive and difficult, especially the implementation using the mechanical 96-channel pipetting aids (instead of a liquid-handling robot). By using such test stations, local health care facilities or NGOs, e.g. together with an academic partner, could establish fast and reliable nucleic acid testing capacity without having to worry about high licensing fees or dependencies on provider-specific supply chains or repair services. In addition, the test station allows easy development of custom tests for a broad class of pathogens by designing and testing new specific RT-LAMP primer sets, e.g. for the detection of arbovirus infections in blood samples. However, the open architecture of the test platform is more susceptible to contamination than commercial closed systems and requires rigorous implementation of strict measures to protect against contamination. Such projects therefore only have a chance if, in addition to the training of personnel and the construction, the long-term operation of the station is also secured by well-funded positions.

Minor issues
- Abstract, first line: rather "capacities".
Re: We corrected the word accordingly on p.1: *"limited diagnostic capacities prevented sentinel testing"* (Changes in magenta).
>> BP: OK

- Second sentence: please specify "alternative" - cheaper, higher throughput, less investments into devices needed…
Re: We revised the sentence accordingly on p.1: *"Here, we describe the set-up of a cost-effective platform in a high-throughput manner,"* (Changes in magenta).

>> BP: OK, but revise grammar: 'that can be employed in a high-throughput manner' o.a.
We revised the grammar.

- Figure 1: the sentence in the legend for D, "Colors and shapes indicate individual patients" should also be mentioned for B. Also, what is the dashed line? Figure 1D is a bit confusing, consider altering the drawing of time line on top a bit and better labeling of figure axis.
Re: We revised the legend for Fig 1B and Fig 1C accordingly on p.8. In Fig 1B, dots on the dashed line indicates that the Ct values of samples collected before and after eating/toothbrushing are the same.

>> BP: OK

- Page 9, what does "SOP1"/"SOP2" refer to?
Re: Sorry for the confusion. SOP means 'Standard operation procedure', both of which are now referenced properly: *see also the corresponding Standard Operation Procedure which is provided as Supplementary File SOP1* resp. *SOP2* (p.9. and p.25.)

>> BP: OK

- Fig. 2C, the colored lines are difficult to see. Consider making them stronger, put them on top of the bundle of brown lines, and make colors more distinguishable.
Re: Fig 2C is showing the differences in absorbance (ΔOD) of each well at 434nm and 560nm during 30-min incubation of the RT-LAMP assay. Here we use the same color code with Fig 2A and 2B to be consistent. Since the positive samples (brown) have similar viral load with the positive controls (green), the curves are overlapping and not clearly distinguishable. While this is not easy to be seen in this figure that shows a screenshot from our user interface, in the user interface itself (Fig EV3), each amplification curve can be highlighted individually by simply clicking on it, in order to distinguish one sample from the other samples and controls. To explain this, we now introduced one additional sentence into the legend to Fig 2C on p.11: *"(C) Time-course analysis of the color change (ΔOD) of the LAMP reaction. Controls as indicated in (A). The plots represent screenshots from our user interface. In the user interface itself (Fig S3), each amplification curve can be highlighted individually by simply clicking on it, in order to distinguish it from the other samples and controls."* (Changes in magenta).

>> BP: OK

- Fig. S2D, add the slope value. Also, the panels of supplementary figure 2 are not addressed in the text.
Re: In the revised manuscript, we added the equation of linear regression to the Fig. S2D (Fig. EV2D in this revised version), and also addressed the figure on p.9: *"To accommodate high numbers of samples, we implemented and validated an automated RNA extraction with a laboratory liquid handling device (Hamilton NGS STAR), which resulted in a time saving of ~55 min (40%) for one sample plate at similar quality (Supplementary Figure EV2A-EV2B, SOP1). To compare the efficiency of the automated RNA isolation protocol with the manual*

RNA isolation protocol, we applied both protocols using a 3-fold serial dilution series of one SARS-CoV-2 gargle sample (Ct = 30. Viral RNA could be detected by RT-qPCR in samples diluted up to 81-fold after either automated or manual RNA isolation. Either of the two protocols showed amplification curves with an interval of 1.5 Ct values for each 3-fold dilution step (Supplementary Figure EV2C). We also evaluated the automated RNA isolation protocol using several different weakly positive samples (Ct > 31), using automated and manual RNA isolation in parallel. The Ct values of isolated RNA from both protocols were in good agreement (Figure EV2D), indicating that automated RNA isolation is robust."
(Changes in magenta).
>> BP: OK. See above cf. ref 1.

- Page 9: the positive controls are not clearly explained. Are the positive controls in Fig. 2 the Zika RNA ones?
Re: Sorry for the confusion. The positive controls are SARS-CoV-2 positive samples, whereas the Zika RNA is used as an internal control that is included in the lysis buffer during RNA isolation. In the revised manuscript, we modified the text on p. 9: *"Per 96-position rack, 92 Micronic tubes with test samples and four controls were included (Figure 2A). Three SARS-CoV-2 positive samples with known concentrations (with Ct value of 29, 32, and 36 respectively) were used as positive controls to evaluate the performance of RNA isolation and RT-LAMP assay. In addition, a negative control (saline) was incorporated to determine intra-assay contaminations."*(Changes in magenta).
>> BP: OK

- "which was higher than what could have been expected for unbiased surveillance testing" - one could also say that an unbiased testing obviously leads to higher values. It is interesting to compare the number with incidences, but this part of the sentence is rather to be omitted.
Re: Thanks for your kind suggestion. This sentence has been deleted in the revised manuscript on p.19.
>> BP: OK

• Variant sequencing: instead of Fig. 5C, rather show which variant was detected at which time point.
Re: Our study protocol approved by the ethics commission required that we anonymize the samples before sequencing, which made it impossible to track them back to individual dates.
>> BP: OK, but I suggest to make that point briefly in the manuscript.
Done: in Methods we now state: "SARS-CoV-2 positive samples were anonymized (requested by the study protocol) and the viral genomes were sequenced using the ARTIC …"

- The symbol for heat inactivation in Fig. 4B is a bit misleading given the information in the methods.
Re: We're sorry for the confusion. In Fig 4B the symbol was meant to indicate the scanning of the sample. We now also included an additional symbol for heat inactivation.
>> BP: OK

2nd Revision - Editorial Decision **9th Mar 2023**

Dear Michael,

I am pleased to inform you that your manuscript has been accepted for publication in EMBO Reports.
We will fast track publish it.

Best regards,

Bernd

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Reporting Checklist for Life Science Articles (updated January 2022)
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures
1. Data
The data shown in figures should satisfy the following conditions:
- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:

- common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;

- are tests one-sided or two-sided?
- are there adjustments for multiple comparisons?
- exact statistical test results, e.g., P values = x but not P values < x;
- definition of 'center values' as median or average;
- definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

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For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Not Applicable	

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Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Reagents and Tools Table

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If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

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Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Yes	Materials and Methods
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the	Yes	Materials and Methods
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Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
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Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Yes	Materials and Methods
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Yes	Materials and Methods, Data Availability Section
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	