**Diagnostic accuracy of Loop mediated isothermal amplification coupled to Nanopore sequencing (LamPORE) for the detection of SARS-CoV-2 infection at scale in symptomatic and asymptomatic populations**

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**Abstract**

Objectives:

Rapid, high throughput diagnostics are a valuable tool, allowing the detection of SARS-CoV-2 in populations, in order to identify and isolate people with asymptomatic and symptomatic infections. Reagent shortages and restricted access to high throughput testing solutions have limited the effectiveness of conventional assays such as reverse transcriptase quantitative PCR (RT-qPCR), particularly throughout the first months of the COVID-19 pandemic. We investigated the use of LamPORE, where loop mediated isothermal amplification (LAMP) is coupled to nanopore sequencing technology, for the detection of SARS-CoV-2 in symptomatic and asymptomatic populations.

Methods: In an asymptomatic prospective cohort, for three weeks in September 2020 health care workers across four sites (Birmingham, Southampton, Basingstoke and Manchester) self-swabbed with nasopharyngeal swabs weekly and supplied a saliva specimen daily. These samples were tested for SARS-CoV-2 RNA using the Oxford Nanopore LamPORE system and a reference RT-qPCR assay on extracted sample RNA. A second retrospective cohort of 848 patients with influenza like illness from March 2020 – June 2020, were similarly tested from nasopharyngeal swabs.

Results: In the asymptomatic cohort a total of 1200 participants supplied 23,427 samples (3,966 swab, 19,461 saliva) over a three-week period. The incidence of SARS-CoV-2 detection using LamPORE was 0.95%. Diagnostic sensitivity and specificity of LamPORE was >99.5% (reducing to ~ 98% when clustered estimation was used) in both swab and saliva asymptomatic samples when compared to the reference RT-qPCR test. In the retrospective symptomatic cohort, the incidence was 13.4% and the sensitivity and specificity were 100%.

Conclusions: LamPORE is a highly accurate methodology for the detection of SARS-CoV-2 in both symptomatic and asymptomatic population settings and can be used as an alternative to RT-qPCR.

**Introduction**

COVID-19, caused by an emergent novel *betacoronavirus* known as SARS-CoV-2; represents a public health emergency. [1].

Rapid detection of infected cases in order to limit transmission, remains challenging as most validated methods utilise reverse transcription quantitative polymerase chain reaction (RT-qPCR) [2] . Although considered the gold standard for diagnosis, RT-qPCR is laborious can be difficult to scale up for mass-testing, and competition for reagents/equipment from many laboratories may lead to widespread reagent shortages. Initially in the outbreak, labs throughout the United Kingdom utilised primers that were designed to target sequences within the RNA dependent polymerase gene (RdRp) [3], however these lacked sensitivity [4]. RT-qPCR Primer sets were introduced that targeted the envelope (*E*), nucleocapsid (*N*) and *ORF1ab genes*, which provided necessary increased sensitivity [5]. [6]

Loop mediated isothermal amplification (LAMP) offers an alternative to RT-qPCR [7]. This reaction typically takes 20-30 minutes which is considerably quicker than PCR. [8]

Nanopore sequencing allows rapid sequencing using protein nanopores embedded in a lipid membrane. [9]. Nanopore sequencing technology allows all the advantages of conventional next generation sequencing technology, especially the capacity to perform very high (limited only by the number of barcodes available) sample multiplexing within the same sequencing run.

LamPORE is a combination of LAMP and Nanopore sequencing, developed by Oxford Nanopore [10] This technology has a theoretical maximum capacity of 15,000 samples per GridIon Mk 1 machine (Oxford, Nanopore) per 24 hours, allowing scalability and high throughput.

Use of alternative sampling strategies, such as saliva, could theoretically increase capacity over swabbing (because of less pressure on the supply chain) and increase compliance because of the less invasive nature of sampling of saliva. [11].

This study aimed to assess the assay performance characteristics of the LamPORE SARS-CoV-2 Detection Assay against the gold standard RT-qPCR for SARS-CoV-2 detection in both symptomatic and asymptomatic populations from multiple independent centres.

**Methods**

**Study design:**

The study consisted of a retrospective and prospective diagnostic accuracy study comparing the performance of LamPORE sequencing of the *ORF1ab*, *N2* and *E* gene targets of SARS-CoV-2 against RT-qPCR of the *ORF1ab* and *N1* gene targets of SARS-CoV-2 [12].

**Participants**

For the prospective study 1200 health care workers at high risk of asymptomatic transmission were recruited as part of a consented National Health Service England and NHS Improvement (NHSE/I) service evaluation in September 2020. Prospective participants performed naso-pharyngeal self-swabs at day 0, 7, 14 and 21 as well as daily saliva sampling for 21 days (Figure 1). Participants were recruited from staff working within five sites: University Hospitals Birmingham NHS Foundation Trust, Birmingham Women’s’ and Children’s’ NHS Foundation Trust, University Hospital Southampton NHS Foundation Trust, Hampshire Hospitals NHS Foundation Trust and Manchester University NHS Foundation Trust. The retrospective study was undertaken by collating surplus sample from patients having diagnostic samples sent to the Public Health England West Midlands laboratory for respiratory panel testing for influenza like illness (ILI) from January 2020 – June 2020 (National Research Ethics Service Committee West Midlands - South Birmingham 2002/201 Amendment Number 4).

**Test methods**

**Sampling**: For swab-based tests, participants underwent self-directed nasopharyngeal swabbing using flocked swabs containing viral transport medium (VTM). For saliva-based tests participants were instructed to dribble at least 1ml of saliva into a universal specimen container without any additive. Samples were tested immediately if returned on the day of testing or if received on Friday then stored for a maximum of 4 days at 4oC then tested.

**Clinical material used for analytical performance:**

To assess the limit of detection and precision, a tenfold dilution series (from 20,000 copies/ml to 0.2 copies/ml) of droplet digital polymerase chain reaction (ddPCR) quantified SARS-CoV-2 was used and tested in triplicate. . A panel of respiratory viruses (Zeptometrix Respiratory Panel R2, Buffalo NY 14202) was used to assess specificity of the LamPORE assay.

**RNA extraction**: For all samples at the Birmingham site, samples were heated to 56oC for a minimum of 30 minutes in order to inactivate live SARS-CoV-2 virus and. RNA extraction was performed. For every batch of RNA extraction performed (95 samples/batch) an RNA extraction control was utilised consisting of heat inactivated SARS-CoV-2 virus grown on Vero-E6 cells (PHE SARS-CoV-2 England reference strain)

**Reference test (RT-QPCR)**: Single step reverse transcription quantitative polymerase chain reaction (RT-QPCR) against the ORF1ab and N1 gene targets of SARS-CoV-2 was carried out using the CerTest ViaSure SARS-CoV-2 real time PCR kit (CerTest Biotech SL, Zaragoza, Spain) according to manufacturer’s instructions for use (IFU) on ThermoFisher QuantStudio 5 or BioMolecular Systems MIC instruments, using 5L of extracted RNA per reaction. [2, 12]

**Comparator test (LamPORE)**: For each sample, 20L of RNA sample underwent amplification and sequencing using the LamPORE technique as per the manufacturers protocol .A proprietary Guppy/VSEARCH/SnakeMake pipeline (algorithmic details as per James et al [13]) that aligned reads to the viral target genes and a human beta-actin (*ACTB*) internal control and reported results in absolute reads per sample per gene.

**Analysis**

**Sample size calculation:**

Sample size was determined pragmatically, based on the incidence seen in the United Kingdom at the time of the study (1%). Sample size was calculated using R code (using R 3.6.3 [14]) from the methodology of Stark et al. [15] for binary diagnostic test outcomes (a=0.05, b=0.90) setting a base sensitivity and specificity of RT-qPCR of 95% and 99% respectively. We aimed to be able to detect a change of sensitivity & specificity of 10% in LamPORE respectively giving a sample size of greater than 9,600 in the prospective cohort.

**Results interpretation:**

The readers of RT-qPCR and LamPORE tests were blinded to any clinical information relating to study participants. For the RT-qPCR reference assay, SARS-CoV-2 was said to be detected if the following conditions were met; amplification of the kit internal control, amplification of either the *ORF1ab* or *N* gene with a cycle threshold of Cycle threshold (CT) < 38, detection of the positive control on the sample plate, detection of the RNA extraction control on the sample plate, and no SARS-CoV-2 specific amplification in the negative control. CT values were calculated automatically using instrument software with automatic baseline setting calculated. All curves were manually inspected by two investigators in order to check for quality and inhibition of reaction.

LamPORE: Aligned read counts were generated via the LamPORE pipeline against *ORF1ab* (labelled AS1), *E1* and *N2* genes, as well as a human *ACTB* gene internal control. Any unaligned reads were marked as “Undetermined”. Samples were called positive if any of the SARS-CoV-2 target genes had > 50 reads/sample, indeterminate if between 20-50 reads and negative if less than 20 reads. ACTB gene counts were not used as part of the calling algorithm but were used to infer sufficient sampling.

Test results were compared using a 2x2 table and standard measures of sensitivity, specificity, positive predictive value and negative predictive value were calculated using R. If results from either the RT-qPCR or LamPORE test were missing or indeterminate then no comparison was made and the sample was removed from the analysis. Standard analyses of variability in diagnostic precision were made, and modification to the analysis was made to carry out clustered analyses of diagnostic precision using clustered logistic regression with a sandwich estimator (Stata 16.1, StataCorp, TX), on the basis that each separate study represented a cluster within the whole and may bias estimates of sensitivity and specificity [16] .

**RESULTS**

**Participants**

For the prospective asymptomatic study, a total of 1200 participants who were at work and reported to be well were recruited across the four sites (Birmingham n=600, Southampton n=200, Basingstoke/Winchester n=200, Manchester n=200). There were no adverse events. Sample flow is shown in Figure 2.

**Analytical performance**

LamPORE reliably detected SARS-CoV-2 to 20 copies/ml of sample. SARS-CoV-2 reads were detected in the 0.2 copies/ml sample but this was below the threshold for calling as positive sample in LamPORE but were not detected via RT-qPCR (Table 1, Figure 3).

Intra- and inter-assay precision was calculated against the *ORF1ab* gene. For intra-assay precision on a single day, the standard deviation of *ORF1ab* was 50 reads with a coefficient of variation (CV) of +/- 2.3% (supplementary table 1). For inter-assay precision across multiple days, the standard deviation of *ORF1ab* was 178 reads with a CV of +/- 7.8% (Supplementary table 2).

For reproducibility for 24 replicates the standard deviation for *ORF1ab* gene was 128 reads with a CV of +/- 3.9% (Supplementary table 3).

For analytical specificity of the LamPORE assay, SARS-Cov-2 was not detected in any of the samples within the respiratory virus panel. In terms of quality control, the median number of reads reported aligned per sample to *ACTB* was 571 reads (range 76-7249). The median ratio of mapped (to SARS-CoV-2 or ACTB) to unmapped reads was a median of 0.71 (IQR -0.63-0.79). There was generally lower levels of reads seen in extracted VTM vs. saliva in *ACTB* (mean 646 vs. 206 reads, p<0.001), ORF1ab (mean 146 vs. 98 reads, p<0.001) and E1 (14 vs. 8 reads, p <0.01) but not in N2 (22 vs. 17 reads, p=0.06).

In order to understand the context in which LamPORE operates, a comparative experiment was carried out examining the sensitivity of our reference RT-qPCR assay vs. flourimetric LAMP (Optigene LAMP kit) vs. LamPORE (supplementary table 4). This showed that LamPORE detected three additional positive samples detected by RT-qPCR that were not detected by RT-LAMP.

**Test results**

All participants

In total 23,427 samples were obtained from all participants, of which 22,401 were from the asymptomatic study and 848 were from the retrospective symptomatic cohort. Both LamPORE (comparator assay) and RT-qPCR (reference assays) were performed on all 23,427 samples (Table 2).

Of the 601 samples positive on LamPORE assay, 477 were also positive and 124 negative by RT-qPCR and comparison to the reference assay and 124 were false positives. Of the 22,826 negative samples, 22,824 were confirmed as negative by the RT-qPCR and there were two samples that were positive by RT-qPCR.

The diagnostic sensitivity (DSe) of the LamPORE assay compared to the Certest ViaSure RT-qPCR assay was 99.58% (95% CI 98.46-99.95%) and the diagnostic specificity (DSp) was 99.46% (95% CI 99.36-99.55%). The positive predictive value (PPV) of the test with a tested population incidence of 2.04% was 79.37% (95% CI 76.34-82.10%) and the negative predictive value calculated with a prevalence of 2.04% (NPV) was 99.99% (95% CI 99.9-100.0%).

When modelled at 1% population prevalence the PPV dropped to 66.24% and at 0.1% population prevalence the PPV was 16.3%. NPV remained at > 99.99% in all population scenarios.

When a clustered regression analysis was performed to calculate clustered sensitivity and specificity on all participants (when the cohorts were used as the cluster definition), sensitivity reduced to 98.52% (95% CI 94.75%-99.82%) and specificity was 97.39% (95% CI 97.14-97.62%. Positive predictive value droped to 22.62% (95% CI 21.03-24.29%) and negative predictive value to 99.99% (95% CI 99.95-100%).

If sensitivity and specificity was calculated as regards to the cohort (n=1200, Supplementary table 5) rather than individual test results, and either a positive or negative test was counted at any point in the 21 days sampling, 66 were positive via LamPORE with an additional two participants detected via QPCR and 1031 testing negative via LamPORE with a further 101 testing negative by QPCR, giving a sensitivity of 97.06% (95% CI 89.78%-99.64%), a specificity of 91.08% (95% CI 89.26-92.67%), a positive predictive value of 39.52% (95% CI 35.07%-44.16%) and a negative predictive value of 99.81% (95% CI 99.25-99.95%)

Asymptomatic cohort

For the asymptomatic cohort (table 2) a total of 22,401 participant samples were tested, with 333 positive (34 swab, 299 saliva) samples being identified of which 210 samples (23 swab, 187 saliva) were true positive and 123 samples (11 swab, 112 saliva) false positive when compared with RT-qPCR. There were 22,068 negative samples in total of which 22,026 samples (3932 swab, 18134 saliva) were true negative and 2 samples (both saliva) false negatives. For this cohort there was a diagnostic sensitivity (DSe) of 99.64% (95% CI 98.0-99.9%), a diagnostic specificity (DSp) of 99.48% (95% CI 99.38-99.57%), a PPV of 69.44% and an NPV of 99.48% (95% CI 99.97-100.00%). For the RT-qPCR assay, the mean *ORF1ab* cycle threshold (Ct) was 17.1 (Range 16.2-37.2) and the *N1* was 14.3 (range 11.0-37.2).

Symptomatic cohort

There was complete agreement between the RT-qPCR and LamPORE assays for 116 positive samples and 752 negative samples, for this cohort resulting in a DSe of 100%, DSp of 100%, PPV of 100% and NPV of 100%. The incidence of SARS-CoV-2 was 13.4% over the study period.

**Variability across time course**

In order to understand the utility of LamPORE across the time course of infection, a single participant who was identified as the beginning of their infection, early in the study with a long time course (5 days) of positivity was studied with daily saliva sampling as per protocol (Figure 4). Initially a high viral load, indicative of a CT value of 19.5 was observed which increased (indicating decreasing viral load) over the five days to 23.5 and then became undetectable at day 6. Only LamPORE *N2* reads were detectable at day 1, but *E1* reads became detectable at day 2 and *ORF1ab* reads at day 4.

In order to understand how the assay performed across the whole spectrum of viral loads, we plotted cycle threshold (Ct) vs. numbers of reads for the ORF1ab and N2 genes (Supplementary figures 1 & 2). There was no strong correlation between number of reads and Ct values, we hypothesied due to the nature of the non-linear amplification techniques used.

**DISCUSSION**

We carried out a very large asymptomatic cohort study of health care workers using a novel technology, LamPORE, comparing it to a reference RT-qPCR assay. We found that LamPORE has high sensitivity and specificity (>99%) in both the asymptomatic and symptomatic populations, directly comparable to RT-qPCR and therefore has comparable predictive ability across a range of use cases in varying levels of population prevalence. We studied a population with a wide range of viral loads as determined by cycle threshold, with LamPORE demonstrating good detection across the range.

LamPORE has the advantage that it is scalable [10] to allow testing of very large population levels because of the use of sample barcoding allowing pooling of up to 3,500 samples on a single GridIon instrument. Due to the increased sensitivity of LAMP as part of the LamPORE system, it gives very high sensitivity for SARS-CoV-2. With combinatorial barcoding as has been adopted in other population level assays [17] on a larger flow cells (e.g. a Promethion flow cell), even greater sample multiplexing may be achievable. Another potential inherent advantage is the ability to multiplex gene targets allowing the detection of multiple respiratory pathogens [18] such as SARS-CoV-2, influenza and respiratory syncytial virus (RSV). It is not known what the upper limit of multiplexing of LAMP primers is, and they are considerably more complex to design than PCR primers [19]. Given the advantages of LAMP in terms of speed of amplification [8] and sensitivity of detection, an exploration of LAMP multiplexing is urgently required. Also, the assay chemistry uses different enzymes and methodologies to PCR, meaning a diversification of supplies and therefore potentially less reagent shortages in a pandemic setting. However, LAMP plus sequencing introduces several steps into the workflow which means that LamPORE becomes inherently “non-linear” i.e. the relationships between the genes amplified and sample viral load is not linear and thus LamPORE may only be used to infer positivity rather than any measure of viral load.

A potential disadvantage of LamPORE is the differing workflow needed to prepare samples, including the LAMP step and library preparation, barcoding then sequencing. This requires more sample preparation steps than a RT-qPCR workflow.

During the testing of the asymptomatic cohort, we observed a number of false positives using LamPORE when compared to the RT-qPCR assay. There are a number of possible explanations for this observation. Firstly, LAMP amplification is more sensitive than PCR amplification [20] and so contamination risk is high but as the laboratories refined the technique, contamination issues seemed to resolve. Secondly, it is feasible that some of the samples are in fact, true positive as demonstrated by the ability of LamPORE to detect spiked, killed virus beyond the limit of detection of RT-qPCR. This may have useful implications for sample pooling [21], as greater sensitivity would allow more samples to be pooled and tested. Finally, the LamPORE protocol requires multiple manual liquid handling steps which can lead to error and increases the number of opportunities for contamination to occur. . We found two false negative samples within the saliva cohort. These had high Ct values (ORF1ab Ct 36.5 and 37.1) in the PCR, and we hypothesise that this may have occurred because of low viral loads as the extracted RNA went through a freeze thaw before it was run on LamPORE.

In conclusion, we have demonstrated the accuracy of LamPORE across a range of population use cases, maintaining a high specificity and sensitivity, reproducibility and limit of detection, as well as working well on saliva samples, making it suitable for the detection of symptomatic and asymptomatic patients with SARS-CoV-2.

**FIGURES**

Figure 1: Graphical representation of recruitment strategy for collecting saliva and swabs. Day of study is shown below thick black horizontal line. Nasopharyngeal swab sampling timings are represented by thick red vertical arrows. Saliva sample timings represented by thin black vertical arrows.



Figure 2: Flowchart showing sample numbers at each stage. Pass = sample passed assay QC; Indeterminate = sample passed QC but did not have a clear result; Fail = sample failed assay QC.



Figure 3: Figure showing *ORF1ab* (black), *E1* (fuchsia) and *N2* (taupe) reads in serial dilution series of SARS-CoV-2 for LamPORE. Detection threshold shown by red dotted line.



Figure 4: Line plot showing data from daily saliva sampling of a single participant reporting symptoms and their cycle threshold for the *N1* gene (red dashed line, left Y axis, reverse order) and read count (right Y axis) for *ORF1ab* (green line), *E1* (purple line) and *N2* (orange line). Days since symptoms began shown on X axis.



TABLES

**Table 1: Dilution series of SARS-CoV-2 virus and LamPORE**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Concentration | ORF1ab reads (median) | E1 Reads(median) | N2 Reads(Median)  | RT-qPCR Result | ORF1ab Ct | N Ct |
| 20000 copies/ml | 6429 | 808 | 2288 | Detected | 10.9 | 14.7 |
| 2000 copies/ml | 1385 | 18 | 602 | Detected | 28.1 | 24.1 |
| 200 copies/ml | 27 | 4 | 82 | Detected | 30.7 | 30.7 |
| 20 copies/ml | 67 | 6 | 979 | Detected | N/D | 33 |
| 2 copies/ml | 0 | 0 | 45 | Not detected | N/D | N/D |
| 0.2 copies/ml | 16 | 0 | 0 | Not detected | N/D | N/B |

**Table 2: Diagnostic assay performance tables for RT-qPCR vs. LamPORE assay for whole cohort, asymptomatic cohort (swabs), asymptomatic cohort (saliva) and retrospective symptomatic cohort. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each cohort are shown at the bottom of each sub-table**

|  |
| --- |
| **Whole cohort** |
|  |  | RT-qPCR |   |   |
|  |  | Positive | Negative |   |
| LamPORE | Positive | 477 | 124 | 601 |
|  | Negative | 2 | 22824 | 22826 |
|  |  | 479 | 22948 |  |
|  |  |  |  |  |
| Sensitivity | Specificity | PPV | NPV  |  |
| 99.58 (95% CI 98.46-99.95%) | 99.46% (95% CI 99.36-99.55%) | 79.37% (95% CI 76.34-82.10%) | 99.99% (95% CI 99.9-100%)  |  |
|  |  |  |  |  |
| **Asymptomatic cohort - swabs** |
|  |  | Positive | Negative |   |
| LamPORE | Positive | 23 | 11 | 34 |
|  | Negative | 0 | 3932 | 3932 |
|  |  | 23 | 3943 |  |
|  |  |  |  |  |
| Sensitivity | Specificity | PPV | NPV  |  |
| 100% (95 CI 85.2-100%) | 99.72% (95% CI 99.5-99.7%) | 67.65% (95% CI 53.7-79.1%) | 100%  |  |
| **Asymptomatic cohort – saliva**  |
|  |  | Positive | Negative |   |
| LamPORE | Positive | 187 | 112 | 299 |
|  | Negative | 2 | 18134 | 18136 |
|  |  | 189 | 18246 |  |
|  |  |  |  |  |
| Sensitivity | Specificity | PPV | NPV  |  |
| 98.9% (95% CI 96.2-99.9%) | 99.4% (95% CI 99.3-99.5%) | 62.5% (95% CI 58.1-66.8%) | 99.9% (95% CI 99.9-100%)  |  |
|  |  |  |  |  |
| **Symptomatic cohort** |
|  |  | Positive | Negative |   |
| LamPORE | Positive | 116 | 0 | 116 |
|  | Negative | 0 | 752 | 752 |
|  |  | 116 | 752 | 868 |
|  |  |  |  |  |
| Sensitivity | Specificity | PPV | NPV  |  |
| 100% (95% CI 96.9%-100%) | 100% (95% CI 99.51-100%) | 100% | 100%  |  |

**SUPPLEMENTARY MATERIALS**

**Supplementary table 1: Intra-assay precision. Standard deviation (SD) *ORF1ab* = 50 reads, coefficient of variation (CV) = +/- 2.3%**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample**  | **Inhibition control value (Unmapped reads)** | ***ORF1ab* reads** | ***E1* gene reads** | ***N2* gene reads** |
| Replicate 1 | 658 | 2207 | 970 | 11 |
| Replicate 2  | 788 | 2176 | 112 | 306 |
| Replicate 3 | 1261 | 2286 | 637 | 782 |
| Replicate 4 | 3126 | 2161 | 159 | 312 |
| Replicate 5 | 565 | 2237 | 11 | 121 |

**Supplementary table 2: Inter-assay precision. Standard deviation (SD) *ORF1ab* = 178 reads, CV = +/- 7.8%**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample**  | **Inhibition control value (Unmapped reads)** | ***ORF1ab* reads** | ***E1* gene reads** | ***N2* gene reads** |
| Replicate 1 | 658 | 2207 | 970 | 11 |
| Replicate 2  | 788 | 2176 | 112 | 306 |
| Replicate 3 | 1261 | 2286 | 637 | 782 |
| Replicate 4 | 3126 | 2161 | 159 | 312 |
| Replicate 5 | 565 | 2237 | 11 | 121 |

**Supplementary table 3: Assay variability. Standard deviation (SD) ORF1ab = 128 reads with CV = +/- 3.9%**

|  |  |  |  |
| --- | --- | --- | --- |
| **Replicate** | **ORF1ab reads** | **E1 reads** | **N2 reads** |
| 1 | 3334 | 1384 | 1342 |
| 2 | 3084 | 445 | 0 |
| 3 | 3500 | 20 | 177 |
| 4 | 3174 | 11 | 60 |
| 5 | 3367 | 60 | 400 |
| 6 | 3013 | 1 | 0 |
| 7 | 3337 | 22 | 352 |
| 8 | 3375 | 14 | 151 |
| 9 | 3183 | 11 | 87 |
| 10 | 3173 | 1045 | 22 |
| 11 | 3190 | 128 | 0 |
| 12 | 3344 | 1293 | 1837 |
| 13 | 3191 | 416 | 664 |
| 14 | 3233 | 821 | 1823 |
| 15 | 3256 | 1792 | 1867 |
| 16 | 3237 | 1779 | 1640 |
| 17 | 3017 | 377 | 142 |
| 18 | 3144 | 1517 | 311 |
| 19 | 3171 | 230 | 365 |
| 20 | 3241 | 574 | 2774 |
| 21 | 3386 | 60 | 1518 |
| 22 | 3493 | 1902 | 16 |
| 23 | 3211 | 82 | 476 |
| 24 | 3206 | 1042 | 1201 |

Supplementary Table 4: Comparison of fluoriomeric LAMP to QPCR to LamPORE in a reference sample set. LAMP “RNA result” = LAMP on extracted RNA, “Direct result” = LAMP direct on crude saliva.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Fluoriometric LAMP** | **RT-QPCR** | **LamPORE** |
| **ID** | **RNA****result** | **Direct result** | **Result (ORF1ab)** | **Result** | **ORF1b reads** | **E1 reads** | **N2 reads** |
| 1 | POS | POS | 20.9 | Positive | 2394 | 961 | 2037 |
| 2 | NEG | NEG | NEG(N=39.1) | Positive | 29 | 35 | 43 |
| 3 | POS | POS | 25.9 | Positive | 1895 | 2070 | 1223 |
| 4 | POS | POS | 22.1 | Positive | 2594 | 182 | 717 |
| 5 | POS | NEG | 34.6 | Positive | 2498 | 310 | 864 |
| 6 | POS | NEG | 35.3 | Positive | 2596 | 27 | 287 |
| 7 | NEG | NEG | NEG (38.2) | Positive | 2274 | 43 | 586 |
| 9 | POS | NEG | 32.8 | Positive | 2176 | 10 | 197 |
| 10 | NEG | NEG | NEG (43.9) | Positive | 2596 | 27 | 287 |
| 11 | NEG | NEG | NEG (42.2) | Positive | 2 | 91 | 31 |
| 12 | POS | POS | 23.2 | Positive | 500 | 211 | 566 |
| 13 | NEG | NEG | NEG | Negative |  |  |  |
| 14 | NEG | NEG | NEG | Negative |  |  |  |
| 15 | NEG | NEG | NEG | Negative |  |  |  |
| 16 | NEG | NEG | NEG | Negative |  |  |  |
| 17 | NEG | NEG | NEG | Negative |  |  |  |
| 18 | NEG | NEG | NEG | Negative |  |  |  |

Supplementary table 5: 2x2 table of LamPORE vs. RT-qPCR when cohort testing rates are used

|  |  |  |
| --- | --- | --- |
|  |  | RT-qPCR |
|  |  | Positive | Negative |
| LamPORE | Positive | 66 | 101 |
| Negative | 2 | 1031 |

**Supplementary Figure 1: Scatter plot of log10 (Ct value for ORF1ab) vs. log10(number of ORF1ab reads) with regression line shown as a dashed line**



**Supplementary figure 2: Scatter plot of log 10(Ct value for N) vs. log10(number of N2 reads) with regression line shown as a dashed line**

**Author contributions:**

Conceptualization ADB, AR, SH, AD, DP

Data curation – ADB, CW,

Formal Analysis – ADB, AR, AW, DB, SH, AD

Funding acquisition ADB

Investigation – all authors

Methodology – ADB, AR, AW, VF, SK, SH, AD, ZD, DP

Project administration SH, AD, ZD, DP

Resources

Software ADB

Supervision ADB, AR, SK, VF

Validation – ADB, AR, SK, VF,

Visualization – ADB, AR

Writing – original draft – all authors

Writing – review & editing – all authors

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**References**

[1] J.J. Zhang, X. Dong, Y.Y. Cao, Y.D. Yuan, Y.B. Yang, Y.Q. Yan, C.A. Akdis, Y.D. Gao, Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China, Allergy 75(7) (2020) 1730-1741.

[2] A. Bosworth, C. Whalley, C. Poxon, K. Wanigasooriya, O. Pickles, E.L. Aldera, D. Papakonstantinou, G.L. Morley, E.M. Walker, A.E. Zielinska, D. McLoughlin, C. Webster, T. Plant, A. Ellis, A. Richter, I.M. Kidd, A.D. Beggs, Rapid implementation and validation of a cold-chain free SARS-CoV-2 diagnostic testing workflow to support surge capacity, J Clin Virol 128 (2020) 104469.

[3] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brunink, J. Schneider, M.L. Schmidt, D.G. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J.L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill 25(3) (2020).

[4] C.B.F. Vogels, A.F. Brito, A.L. Wyllie, J.R. Fauver, I.M. Ott, C.C. Kalinich, M.E. Petrone, A. Casanovas-Massana, M. Catherine Muenker, A.J. Moore, J. Klein, P. Lu, A. Lu-Culligan, X. Jiang, D.J. Kim, E. Kudo, T. Mao, M. Moriyama, J.E. Oh, A. Park, J. Silva, E. Song, T. Takahashi, M. Taura, M. Tokuyama, A. Venkataraman, O.E. Weizman, P. Wong, Y. Yang, N.R. Cheemarla, E.B. White, S. Lapidus, R. Earnest, B. Geng, P. Vijayakumar, C. Odio, J. Fournier, S. Bermejo, S. Farhadian, C.S. Dela Cruz, A. Iwasaki, A.I. Ko, M.L. Landry, E.F. Foxman, N.D. Grubaugh, Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT-qPCR primer-probe sets, Nat Microbiol 5(10) (2020) 1299-1305.

[5] L. Penarrubia, M. Ruiz, R. Porco, S.N. Rao, M. Juanola-Falgarona, D. Manissero, M. Lopez-Fontanals, J. Pareja, Multiple assays in a real-time RT-PCR SARS-CoV-2 panel can mitigate the risk of loss of sensitivity by new genomic variants during the COVID-19 outbreak, Int J Infect Dis 97 (2020) 225-229.

[6] M. Park, J. Won, B.Y. Choi, C.J. Lee, Optimization of primer sets and detection protocols for SARS-CoV-2 of coronavirus disease 2019 (COVID-19) using PCR and real-time PCR, Exp Mol Med 52(6) (2020) 963-977.

[7] T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, T. Hase, Loop-mediated isothermal amplification of DNA, Nucleic Acids Res 28(12) (2000) E63.

[8] V.L. Dao Thi, K. Herbst, K. Boerner, M. Meurer, L.P. Kremer, D. Kirrmaier, A. Freistaedter, D. Papagiannidis, C. Galmozzi, M.L. Stanifer, S. Boulant, S. Klein, P. Chlanda, D. Khalid, I. Barreto Miranda, P. Schnitzler, H.G. Krausslich, M. Knop, S. Anders, A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples, Sci Transl Med 12(556) (2020).

[9] N.J. Loman, J. Quick, J.T. Simpson, A complete bacterial genome assembled de novo using only nanopore sequencing data, Nat Methods 12(8) (2015) 733-5.

[10] P. James, D. Stoddart, E.D. Harrington, J. Beaulaurier, L. Ly, S.W. Reid, D.J. Turner, S. Juul, LamPORE: rapid, accurate and highly scalable molecular screening for SARS-CoV-2 infection, based on nanopore sequencing, medRxiv (2020).

[11] J. Zhu, J. Guo, Y. Xu, X. Chen, Viral dynamics of SARS-CoV-2 in saliva from infected patients, J Infect 81(3) (2020) e48-e50.

[12] CerTest, VIASURE SARS-CoV-2 Real Time PCR Detection Kit. <<https://www.certest.es/wp-content/uploads/2020/03/Coronavirus_Type_II_CE_EN.pdf>>, 2020 (accessed 03/12/2020.).

[13] P. James, D. Stoddart, E.D. Harrington, J. Beaulaurier, L. Ly, S.W. Reid, D.J. Turner, S. Juul, LamPORE: rapid, accurate and highly scalable molecular screening for SARS-CoV-2 infection, based on nanopore sequencing, medRxiv (2020) 2020.08.07.20161737.

[14] R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>.>, 2020).

[15] M. Stark, A. Zapf, Sample size calculation and re-estimation based on the prevalence in a single-arm confirmatory diagnostic accuracy study, Stat Methods Med Res 29(10) (2020) 2958-2971.

[16] T.S. Genders, S. Spronk, T. Stijnen, E.W. Steyerberg, E. Lesaffre, M.G. Hunink, Methods for calculating sensitivity and specificity of clustered data: a tutorial, Radiology 265(3) (2012) 910-6.

[17] J.L. Schmid-Burgk, D. Li, D. Feldman, M. Słabicki, J. Borrajo, J. Strecker, B. Cleary, A. Regev, F. Zhang, LAMP-Seq: Population-Scale COVID-19 Diagnostics Using a Compressed Barcode Space, bioRxiv (2020) 2020.04.06.025635.

[18] W.S. Jang, D.H. Lim, J. Nam, D.C. Mihn, H.W. Sung, C.S. Lim, J. Kim, Development of a multiplex isothermal amplification molecular diagnosis method for on-site diagnosis of influenza, PLoS One 15(9) (2020) e0238615.

[19] B. Jia, X. Li, W. Liu, C. Lu, X. Lu, L. Ma, Y.Y. Li, C. Wei, GLAPD: Whole Genome Based LAMP Primer Design for a Set of Target Genomes, Front Microbiol 10 (2019) 2860.

[20] Y. Bao, Y. Jiang, E. Xiong, T. Tian, Z. Zhang, J. Lv, Y. Li, X. Zhou, CUT-LAMP: Contamination-Free Loop-Mediated Isothermal Amplification Based on the CRISPR/Cas9 Cleavage, ACS Sens 5(4) (2020) 1082-1091.

[21] J. Garg, V. Singh, P. Pandey, A. Verma, M. Sen, A. Das, J. Agarwal, Evaluation of sample pooling for diagnosis of COVID-19 by real time-PCR: A resource-saving combat strategy, J Med Virol (2020).