Department of Health & Social Care

Technical validation by the Scientific Validation and Assurance Group: Rapid evaluation of Oxford Nanopore LamPORE (RNA formats)

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Contents

Executive Summary

1. The LamPORE assay was evaluated according to the published Technical Validation Group protocol (https://www.gov.uk/government/publications/assessment-and-procurement-of-coronavirus-covid-19-tests/coronavirus-covid-19-serology-and-viral-detection-testing-uk-procurement-overview).

2. A collaboration across 5 NHS Trusts and 2 University partners who evaluated LAMP based molecular testing technologies (including Oxford Nanopore LamPORE), in an NHS asymptomatic staff pilot study.

3. As part of this, we also investigated the accuracy of LamPORE on symptomatic patients who had undergone previous respiratory pathogen testing in a “real-world” situation using RNA from samples sent to Public Health West Midlands for testing of patients with Influenza like illness.

4. The LamPORE assay was assessed on saliva and swab samples from asymptomatic participants in the setting of RNA extraction when the underlying disease incidence was 1.99%.

5. The LamPORE assay on swabs in asymptomatic patients, with RNA extraction, returned a sensitivity of 99.57% (95% CI 98.46-99.99%) and the positive predictive value was 78.91% (95% CI 75.84-81.69%). Specificity was 99.40% (95% CI 99.28-99.50%) and negative predictive value was 99.99% (95% CI 99.96%-100.0%).

6. The LamPORE assay on saliva in asymptomatic patients returned a sensitivity of 98.94% (95% CI 96.23-99.87%) and the positive predictive value was 62.54% (95% CI 58.11-66.77%). Specificity was 99.39% (95% CI 99.26-99.49%) and negative predictive value was 99.99% (95% CI 99.96%-100.0%).

7. The LamPORE assay on swab samples in symptomatic patients, with RNA extraction returned a sensitivity of 100% (95% CI 85.18-100%) and a specificity of 100%.

8. As experience with the assay grew, the SOP was refined and earlier contamination issues that had led to false positives were overcome.

9. LamPORE in the RNA format demonstrates equivalent sensitivity and specificity to “gold standard” RT-qPCR with the advantage of enhanced multiplexing (768 samples per flow cell), which provides additional capacity for winter respiratory syndrome testing with significantly higher throughput than some existing technologies (96 samples per plate).

Background

1. The importance of testing, isolation and contact tracing for control of SARS-CoV-2 transmission has been highlighted by the World Health Organisation (WHO) as a critical intervention to prevent the spread of infection and ensuing morbidity and mortality from COVID-19. International efforts have largely focused on the detection of infection in symptomatic individuals as well as evolving clinical testing systems to detect those with asymptomatic or pre-symptomatic (subsequently referred to collectively as asymptomatic) infection that may still be infectious to others.

2. LamPORE combines Loop-mediated isothermal amplification (LAMP) and nanopore sequencing to provide a highly scalable, multi-gene assay for the detection of SARS-CoV-2. LAMP is a single-tube technique for the amplification of DNA and a low-cost, rapid alternative to RT-PCR. Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) combines LAMP with a reverse transcription step to allow the detection of RNA. Target sequence is amplified at a constant temperature. Typically, 4 different primers are used to amplify 6 distinct regions on the target gene, which increases specificity. Additional pairs of "loop primers" can further accelerate the reaction. The amount of amplified product produced in LAMP is considerably higher than PCR-based amplification, due to the use of multiple primer sets.

3. The technology of choice for this provision to date has been reverse transcription polymerase chain reaction testing (RT-qPCR), typically provided in central laboratory facilities, with a sample receipt to result time of >24hours and reliance on international supply chains for a singular testing system. Considering these challenges, alternative technology approaches are being evaluated, including other forms of nucleic acid amplification (i.e. LAMP) and protein-based methods (i.e. Lateral Flow Devices) for viral antigen detection, potentially providing diversification to the overall supply chain and options for an integrated testing strategy. These additional testing assets have also been considered in the context of other components of the wider end to end testing system, including the source and ease of sample collection and frequency of provision (saliva vs swab), RNA extraction requirements, and efficiency of returning results from a testing system into a clinical system for full maximal clinical utility, public health reporting and intervention requirements.

4. LAMP is an isothermal nucleic acid amplification technique, in contrast to the polymerase chain reaction (PCR) technology, in which the reaction is carried out with a series of alternating temperature steps or cycles, isothermal amplification is carried out at a constant temperature, and does not require a thermal cycler.

5. This report outlines the technical validation undertaken in conjunction with the NHS Test and Trace Technical Validation Group and, given the need to prevent nosocomial spread and maintain NHS capacity and capability, it was conducted in collaboration with NHS Trusts and University partners as a real-world evidence study.

Technical Validation

The Technical and Validation function was established under Test and Trace, inclusive of NHS and PHE experts and working closely with Medicines and Healthcare Products Regulatory Agency (MHRA) and research bodies. The Technical and Validation function considers manufacturers of SARS-CoV-2 (COVID-19) tests for viral detection (including LAMP technologies) and registers their interest in the national procurement process if their test meets, or are intended to meet the requirements of a relevant MHRA Target Product Profiles (TPPs).

Assay description and intended purpose

1. LamPORE test is CE marked for use on the GridION X5 device. Each GridION X5 device has five flow cells (a MinION is one flowcell). LamPORE is a test produced by Oxford Nanopore consisting of three key stages:

   The assay uses reverse-transcription coupled to loop-mediated isothermal amplification (RT-LAMP) to amplify three highly conserved genes in SARS-CoV-2 positive samples (E1 gene, N2 gene, ORF1ab) and a negative control gene ACTB (human beta actin)

   Samples are given a molecular barcode so that multiple samples can be analysed together

2. These are then sequenced on the GridION X5 device, and analysed.

3. In order for LAMP to be combined with Nanopore sequencing technologies, several modifications to the LAMP protocol have been made. Each sample is uniquely identified in a 96 well plate by the addition of a short nucleotide sequence (8 letter code) to the forward inner primer (FIP). Secondly, a library pooling and additional identification step, using nucleotide barcodes, must be performed to allow between 96-768 samples to be pooled onto each MinION flowcell.

4. Samples undergo Nanopore sequencing for one hour, and then sequence data is aligned via a custom algorithm to the SARS-CoV-2 genome, producing absolute read counts for the ORF1ab, N2 and E1 genes, as well as an ACTB (human beta actin) internal control. A positive is defined as any of the target genes having greater than 50 reads per sample, indeterminate being between 30-50 reads and negative anything below this. Samples are called as invalid if there are < 50 ACTB reads detected.

5. The validation was performed on nasopharyngeal/oropharyngeal swabs and saliva.

6. Swabs were collected in viral transport medium (Virocult). Saliva was collected in universal plastic tubes. The samples were all collected in clinical settings.

7. All the equipment required to perform the test is supplied by the manufacturer except for a plate centrifuge, thermal cycler, pipettes, magnetic stand, laboratory consumables, saliva collection containers and nasopharyngeal/oropharyngeal swabs

Performance characteristics

Analytical Sensitivity of SARS COV-2 targets

The analytical sensitivity (ASe) for the LamPORE assay was evaluated using a blinded panel of guanidine isothiocynate inactivated SARS-CoV-2 virus grown on Vero E6 cells and quantified by droplet digital PCR to 20000 copies/ml. The CerTest ViaSure SARS-CoV-2 multiplex RT-qPCR assay was used as a direct comparator. LamPORE successfully detected SARS-CoV-2 to a detection threshold of 20 copies/ml (Table 1).

Table 1. Analytical sensitivity - limit of detection of LamPORE vs. reference standard

	Lampore	**Lampore**	**Lampore**	**Lampore**	**ViaSure qPCR**	**ViaSure qPCR**	**ViaSure qPCR**
Concentration (copies/ml)	Detected	ORF1ab reads	E1 reads	N2 reads	Detected	ORF1ab CT	N1 CT
20000	Positive	6429	808	2288	Positive	19.4	23.0
2000	Positive	1385	18	602	Positive	21.8	22.7
200	Positive	27	4	82	Positive	30.2	27.3
20	Positive	67	6	979	Positive	33.3	28.7
2	Negative	0	0	0	Equivocal	-	29.8
0.2	Negative	16	0	0	Negative	-	-

Precision and robustness

1. Intra-assay repeatability and inter-assay reproducibility was carried out using quantified inactivated live virus (as previously quantified by droplet digital PCR). A series of 5 replicates of the same sample undertaken on the same day is shown in Table 2. For intra-assay precision the Standard Deviation (SD) was 50 reads with a Coefficient of Variation (CV) of +/- 2.3% (A satisfactory assay has a CV < 10%) (Table 2).

Table 2. Intra-Assay precision

Sample	Inhibition control value (Unmapped reads)	ORF1ab	E1 gene	N2 gene
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

2. For inter-assay precision the same sample was analysed on multiple days and is seen in Table 3. The SD was 178 reads with a CV of +/- 7.8%. Satisfactory assay performance has a CV < 15%.

Table 3. Inter-Assay precision

Sample	Inhibition control value (Unmapped reads)	ORF1ab	E1 gene	N2 gene
Sample 1 (day 1)	796	2269	821	207
Sample 1 (day 2)	1248	2359	93	93
Sample 1 (day 3)	564	2007	124	219
Sample 1 (day 4)	3747	2292	1541	510

Sample	Inhibition control value (Unmapped reads)	ORF1ab	E1 gene	N2 gene
Sample 1 (day 5)	1380	2495	1687	1547

3. Quantified SARS-CoV-2 virus (via droplet digital PCR) was used to input 20000 copies/ml of extracted SARS-CoV-2 RNA into the LamPORE assay for 24 replicates in order to understand reproducibility. Standard deviation was 128 reads with a CV of +/- 3.9% (Table 4).

Table 4. Reproducibility assessment for LamPORE

Replicate	Concentration	ORF1ab	E1	N2
1	20000 copies/ml	3334	1384	1342
2	20000 copies/ml	3084	445	0
3	20000 copies/ml	3500	20	177
4	20000 copies/ml	3174	11	60
5	20000 copies/ml	3367	60	400
6	20000 copies/ml	3013	1	0
7	20000 copies/ml	3337	22	352
8	20000 copies/ml	3375	14	151
9	20000 copies/ml	3183	11	87
10	20000 copies/ml	3173	1045	22
11	20000 copies/ml	3190	128	0
12	20000 copies/ml	3344	1293	1837

Replicate	Concentration	ORF1ab	E1	N2
13	20000 copies/ml	3191	416	664
14	20000 copies/ml	3233	821	1823
15	20000 copies/ml	3256	1792	1867
16	20000 copies/ml	3237	1779	1640
17	20000 copies/ml	3017	377	142
18	20000 copies/ml	3144	1517	311
19	20000 copies/ml	3171	230	365
20	20000 copies/ml	3241	574	2774
21	20000 copies/ml	3386	60	1518
22	20000 copies/ml	3493	1902	16
23	20000 copies/ml	3211	82	476
24	20000 copies/ml	3206	1042	1201

Analytical specificity (Interferences and cross-reactions)

1. Analytical specificity (ASp) was determined using the NATtrol™ Respiratory Verification Panel 2 (ZeptoMetrix Corporation, New York, United States) containing pathogens causing indistinguishable clinical signs to COVID-19 (n=22). No cross reactivity was observed in LamPORE to any respiratory pathogen.

   Table 1: PANEL MEMBERS

Target	**Result**
Parainfluenza2	Negative
Negative control	Negative
Influenza B Florida 02 06	Negative
Coronavirus NL63	Negative
B.parapertussis A747	Negative
Parainfluenza4	Negative
Influenza ah1 a/newcal/20/99	Negative
Parainfluenza 3	Negative
Coronavirus HKU-1	Negative
B.parapertussis A639	Negative
Metapneumovirus 9 - peru6-2003	Negative
Rhinovirus type 1a	Negative
Adenovirus 31	Negative
Parainfluenza 1	Negative
Adenovirus 1	Negative
M.pneumoniae M129	Negative
Coronavirus 229E	Negative
RSV-A2	Negative
InfluenzaA H1N1pdm	Negative
Coronavirus OC43	Negative

Target	Result
Influenza AH3	Negative
C.pneumioniae CWL-029	Negative
Adenovirus3	Negative
NATtrolMERS-CoV stock	Negative

Table 5. Specificity assessment for LamPORE

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives)

1. Samples selected for the validation were appropriate to the intended use case scenarios, including, low medium and high viral load samples. This dynamic range permits a rigorous evaluation of diagnostic sensitivity (Table 6).

Table 6. Range of viral loads for validation samples

Gene	Mean (CT)	Min (CT)	Max (CT)
ORF1ab	17.1	16.2	37.2
N1	14.3	11.0	37.2
IC	19.8	22.8	25.5

2. Diagnostic sensitivity: Confirmed clinical samples from patients (positive RT-qPCR result) were compared. The CT values or equivalent for both the assessed and comparator assays were included in the submitted validation data.

   34 positive swabs analysed by LamPORE;

   299 positive saliva samples analysed by LamPORE;

   103 positive retrospective respiratory swab samples analysed by LamPORE;

3. Diagnostic specificity: Confirmed clinical samples from patients (negative RT-qPCR result) were used. The CT values or equivalent for both the assessed and comparator assays were included in the submitted validation data.

   3,943 negative swabs analysed by LamPORE;

   18,246 negative saliva samples analysed by LamPORE

   745 negative retrospective respiratory samples analysed by LamPORE;

4. The diagnostic sensitivity (DSe) and specificity (DSp) (diagnostic precision) derived from the above samples are as follows:

5. All Samples

For LamPORE, diagnostic sensitivity (DSe) was calculated as 99.58% (95% CI 98.46-99.95%) and positive predictive value was 79.37% (95% CI 76.34-82.10%). Underlying disease incidence was 2.04% (Table 7).

For LamPORE, diagnostic specificity (DSp) was calculated as 99.46% (95% CI 99.36-99.55%) and negative predictive value was 99.99% (95% CI 99.97%-100.0%).

Table 7. Diagnostic precision on all samples

		Comparator Assay Result	**Comparator Assay Result**	**Total**
Assessed Assay		Positive	Negative	
Assessed Assay	Positive	477	124	601
Assessed Assay	Negative	2	22824	22826
Assessed Assay	Total	479	22948	

6. Swab (asymptomatic) samples

For LamPORE, diagnostic sensitivity (DSe) was calculated as 100% (95% CI 85.18-100%) and positive predictive value was 67.65% (95% CI 53.68-79.05%). Underlying disease prevalence was 0.58% (Table 8).

For LamPORE, diagnostic specificity (DSp) was calculated as 99.72% (95% CI 99.50-99.86%) and negative predictive value was 100%.

Table 8. Diagnostic precision and swab samples

		Comparator Assay Result	**Comparator Assay Result**	**Total**
Assessed Assay		Positive	Negative	
Assessed Assay	Positive	23	11	34
Assessed Assay	Negative	0	3932	3932
Assessed Assay	Total	23	3943	3966

7. Saliva (asymptomatic) samples

For LamPORE, diagnostic sensitivity (DSe) was calculated as 98.94% (95% CI 96.23-99.87%) and positive predictive value was 62.54% (95% CI 58.11-66.77%). Underlying disease prevalence was 1.03% (Table 9).

For LamPORE, diagnostic specificity (DSp) was calculated as 99.39% (95% CI 99.26-99.49%) and negative predictive value was 99.99% (95% CI 99.96%-100%).

Table 9. Retrospective (symptomatic) samples

		Comparator Assay Result	**Comparator Assay Result**	**Total**
Assessed Assay		Positive	Negative	
Assessed Assay	Positive	187	112	299
Assessed Assay	Negative	2	18134	18136
Assessed Assay	Total	189	18246	18435

8. Retrospective (symptomatic) samples

9. The symptomatic sample set was collected from patients undergoing diagnostic testing in the laboratories of Public Health West Midlands (n=848) who underwent swabbing for an influenza like illness (ILI) in the period March 2020-June 2020 or in the Milton Keynes Lighthouse Laboratory where both swabs and saliva was taken (n=20)

10. For LamPORE, diagnostic sensitivity (DSe) was calculated as 100% (95% CI 96.48-100%) and positive predictive value was 100%. Underlying disease prevalence was 12% (Table 10).

11. For LamPORE, diagnostic specificity (DSp) was calculated as 100% and negative predictive value was 100% (95% CI 99.51-100%).

12. A significant increase in accuracy was observed in these samples as they were analysed at the end of project where the maximum experience with LamPORE had been gained.

13. Table 10. Diagnostic precision on retrospective samples

		Comparator Assay Result	**Comparator Assay Result**	**Total**
Assessed Assay		Positive	Negative	
Assessed Assay	Positive	116	0	116

		Comparator Assay Result	Comparator Assay Result	Total
Assessed Assay	Negative	0	752	752
Assessed Assay	Total	116	752	868

Conclusions

1. The technical performance of the LamPORE assay reveals a sensitivity of 99.57% (95% CI 98.46-99.99%) and specificity of 99.40% (95% CI 99.28-99.50%). This provides a comparable analysis to current standard of care RT-qPCR tests on RNA extracted swabs (in asymptomatic settings). This supports the introduction of this alterative nucleic acid amplification technology into the testing repertoire as a diagnostic tool.

2. When the technology is evaluated in the setting of saliva samples, using RNA extraction the sensitivity is 98.94% (95% CI 96.23-99.87) and specificity 99.39% (95% CI 99.26-99.49%) across all samples tested. The performance of RT-LAMP demonstrated excellent specificity and sufficient sensitivity to be used as part of the infection control strategy for SARS-CoV-2.

3. In an attempt to understand the accuracy of LamPORE in a high prevalence setting, using retrospective samples from Public Health England influenza like illness (ILI) plates, the sensitivity was shown to be 100% with a specificity of 100%. This section of the project was carried out at the end when the testing laboratory had the maximum experience with LamPORE and the high accuracy was probably as a consequence of the experience that the participating laboratories had gained in the technique.

4. Early in the study we noted issues with contamination causing spurious false positives in saliva samples, which we found were due to the viscosity of saliva making automated liquid handling challenging. By modifying the provided SOP from Oxford Nanopore we found these contamination issues were largely remedied. The technique is vulnerable to contamination and so very careful attention must be paid to the SOP. Contamination could be identified easily by visually examining the datasets, which may allow development of machine learning algorithms to identify this phenomenon.

5. Operation of LamPORE (and any LAMP technique) requires a dedicated lab set up according to a standardised SOP in order to prevent problems with contamination. Additionally, LIMS provision allowing data exchange between the GridION instrument and LIMS systems is improving.

6. The key advantage of LamPORE, as part of a testing regime for both saliva and swabs, is its capability to offer high throughput testing of SARS-CoV-2 for both asymptomatic or symptomatic testing. The recommended use case is as a mass testing tool for large asymptomatic populations.

7. The aim of an asymptomatic healthcare worker testing service is to detect unsuspected COVID-19 and thereafter to reduce the risk of SARS-CoV-2 transmission to other workers, to patients and to workers' families. The vulnerability of patients is a

key issue here, and such testing offers the opportunity to support infection control measures that are already in place. An early detection system could also support staff workforce resilience and so maintain NHS capacity.

8. Within a use case scenario of regular, frequent, testing of asymptomatic individuals within a population (e.g. healthcare worker staff group) for SARS-CoV-2, the ability to rapidly and accurately identify individuals with high levels of infective virus to facilitate their prompt isolation will be an important contribution to reducing transmission of infection. The LamPORE assay in the RNA mode has been successfully applied in this use case to populations of healthcare workers with the aim of curtailing transmission between staff and to patients within healthcare settings. The NHS asymptomatic staff saliva testing pilot successfully facilitated daily on-site testing as an additional test to support SARS-CoV-2 detection.

9. LamPORE has comparable sensitivity and specificity to the current gold standard assay but with a considerably higher multiplexing capacity, with the ability to incorporate other gene targets such as Influenza and Respiratory Syncytial Virus (RSV) which can form part of a winter respiratory illness screening programme.

10. Further development of the LamPORE technology should consider operation in the Direct mode, challenges of scaling across differently sized organisations, the adoption of a new technology in clinical laboratories and systems that are unaccustomed to this nucleic acid amplification approach and the information technology integration that is needed to support a quality assured clinical service.

11. The diversification and stratification of the SARS-CoV-2 testing technologies and their applied stratification by defined use case testing scenarios in either clinical diagnostic or public health population-based interventions promise to provide additional capacity and capability to the emerging use case scenarios of testing as a public health intervention measure, however the scaling and other operational challenges are well recognised.

Additional Data

1. Local Verification reports

The LamPORE assay has been locally verified in 4 sites, with the activities of each site listed below:

Site	RT-LAMP Evaluations
Institute of Cancer & Genomic Science University of Birmingham (samples from University Hospitals Birmingham NHS Foundation Trust)	-Optimisation of LamPORE assay and generation of new standard operating procedure (SOP) -Limit of detection and analytical sensitivity (ASe) and specificity (ASp). -RNA LamPORE as a screening tool in asymptomatic patients using swabs -RNA LamPORE as a screening tool in asymptomatic patients using saliva -RNA LamPORE in a high prevalence symptomatic cohort
Hampshire Hospital Foundation Trust (Basingstoke and North Hampshire Hospitals site)	-RNA LamPORE as a screening tool in asymptomatic patients using swabs -RNA LamPORE as a screening tool in asymptomatic patients using saliva
University Hospital Southampton (RNA extraction) / University of Southampton (Testing)	-RNA LamPORE as a screening tool in asymptomatic patients using saliva
Manchester University Foundation Trust	-RNA LamPORE as a screening tool in asymptomatic patients using swabs -RNA LamPORE as a screening tool in asymptomatic patients using saliva

2. Sample provenance by site

The sample provenance is illustrated by site and by methodology in Appendices 1-2.

Appendix 1: Sample provenance RNA LamPORE Swab

Appendix 2: Sample provenance RNA LamPORE Saliva

Appendix 1: Sample provenance RNA LamPORE on swab samples

University Hospitals Birmingham NHS Foundation Trust/University of Birmingham

		Pos	Neg	Total
RNA	Pos	18	0	18
<38	Neg	8	1930	1938
Swab		26	1930	

University of Birmingham (PHE Influenza like illness plates)

		Pos	Neg	Total
RNA	Pos	103	0	103
<38	Neg	0	745	745
Swab		103	745	

Hampshire Hospitals Foundation Trust/Royal Hampshire County Hospital

		Pos	Neg	Total
RNA	Pos	3	0	3
<38	Neg	3	1555	1558
Swab		6	1555	

Public Health Lab Manchester/CMFT

		Pos	Neg	Total
RNA	Pos	2	0	2
<38	Neg	0	447	447
Swab		2	447	

Appendix 2: Sample provenance RNA LamPORE on saliva samples

University Hospitals Birmingham NHS Foundation Trust/University of Birmingham

		Pos	Neg	Total
RNA	Pos	26	1	27
<38	Neg	106	9411	9517
Swab		132	9412	

University of Birmingham (PHE Influenza like illness plates)

		Pos	Neg	Total
RNA	Pos	13	0	13
<38	Neg	0	890	890
Swab		13	890	

Hampshire Hospitals Foundation Trust/Royal Hampshire County Hospital

		Pos	Neg	Total
RNA	Pos	129	0	129
<38	Neg	6	5523	5529
Swab		135	5323	

Public Health Lab Manchester/CMFT

		Pos	Neg	Total
RNA	Pos	19	1	20
<38	Neg	0	2310	2310
Swab		19	2311	

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