

RT-LAMP in the screening of NHS staff for COVID –19

Abu Camara¹ Jacqueline Smith¹

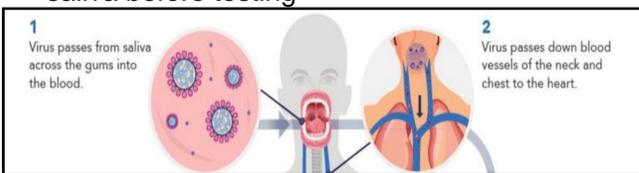
1.LAMP Testing Laboratory, Black Country Pathology Services, Wolverhampton, West Midlands, WV10 0QP.

INTRODUCTION

The recent pandemic and subsequent lockdowns have been caused by a new highly virulent strain of severe acute respiratory syndrome coronavirus 2 (SARS COV-2),

Loop mediated isothermal amplification (LAMP) was designed to overcome some of the limitations of molecular and serological DNA testing such as PCR, this has been done in several ways;

1. Relative low cost of set up
2. Easy training of semi qualified staff
3. RNA does not need to be separated from saliva before testing



(Figure 1) Infection route of covid-19

The use of saliva in LAMP testing is one of its best features, this is due to the fact in early infection the viral load is the highest in the saliva allowing for detection of the virus in asymptomatic staff before they come into contact with patients. LAMP uses four to six primers that target and recognize six to eight distinct regions of a target sequence allowing for sensitive, accurate and rapid amplification facilitating a quick turnaround time of 30 minutes for result .

METHODS

Asymptomatic staff from the West Midlands NHS trust sign up for routine testing and send in a 2ml test tube saliva sample, a 50ul saliva sample is aliquoted from the test tube into a microcentrifuge tube containing 50ul of rapilyze, this mixture is then vortexed and heated for 2 minutes at 98°C, once heated 5ul of the mixture is aliquoted into genie strip wells containing a reaction mix of specialised master mix and primer at a 17.5ul to 2.5ul ratio. The genie strips are then placed into a OptiGene (Horsham, UK) Genie HT where they are heated at 65°C for 17 minutes, if viral RNA is present the turbidity of the genie well will change and be detected through florescence.

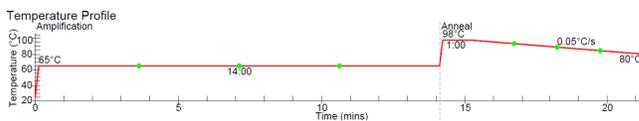


Figure 2 displays the temperature profile of the Genie HT as it runs, first it heats to 65°C for 17 minutes for the amplification phase and then rapidly raises the temperature to 98°C and slowly reduces it to 80°C over 10 minutes allowing for annealing.

Genie® embedded software (OptiGene Ltd., Horsham, UK) was utilised to analyse RT-LAMP results and define thresholds for result calling. All RT-LAMP reactions were performed at least in duplicate, and a sample was considered positive when a time to positivity (tp) was observed in at least one replicate with amplification above 5000 fluorescence points and had an anneal temperature of between 81.50 °C and 84.05 °C with a derivative above 2500 F/°C. Table 2 shows the temperature annealing graph displayed by the Genie HT, the bullet points indicate a positive test showing they satisfy the above stated requirements.

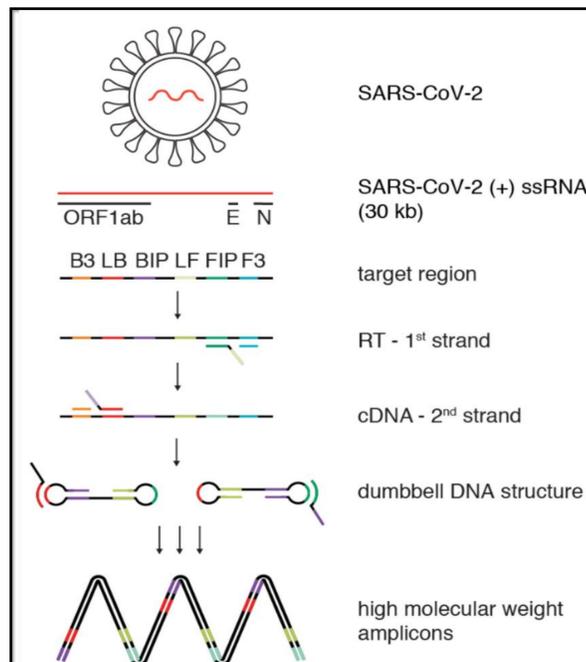


Figure 3 Displays the single stranded positive sense SARS-CoV-2 viral RNA showing the target primer regions after the reverse transcription of the RNA into DNA, these primers bind to the cDNA and form dumbbell DNA structures that are amplified by DNA polymerase to produce a high molecular weight amplicon that can be detected by a Genie analyser.

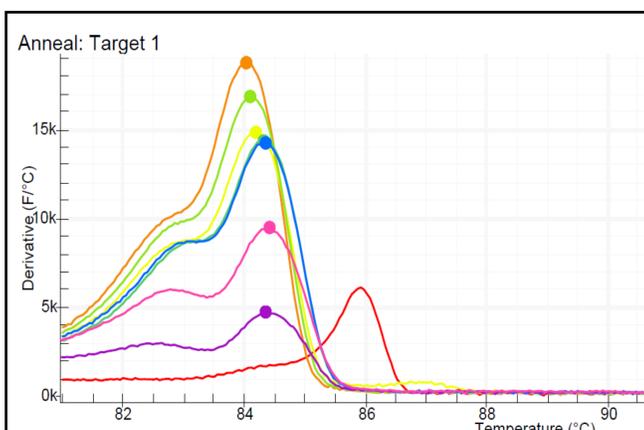
RESULTS

From 5th July to 10th December we saw a positivity rate of 1.57 in every 10,000 samples. From 10th December to 31st January this rose to 42 per 10,000 which was due to the surge in cases from Omicron variant.

Typical results of the Tp value and anneal curve are seen in table 1 below and the anneal curve is seen in table 2.

SAMPLE NO	TP VALUE	ANNEAL CURVE	ORF1ab	Ngene
1	08:01	83.81	17.07	18.66
2	08:21	83.84	19.83	28.68
3	09:25	84.29	18.45	19.08
4	07:51	83.68	16.86	18.04
5	09:28	84.54	21.45	22.25
6	09:41	84.21	21.38	23.22
7	09:00	84.10	23.59	25.40
8	10:46	84.55	11.31	13.17
9	08:20	84.00	14.17	14.03
10	08:37	84.35	19.65	21.20
11	10:52	83.62	18.97	21.05
12	10:17	84.35	22.98	24.82
13	08:33	83.83	21.95	22.55
14	10:47	84.38	26.85	20.32
15	06:45	84.33	12.06	13.82
16	06:42	83.90	10.64	12.23
17	09:08	83.96	20.05	21.97
18	10:24	83.83	23.96	25.11
19	07:41	83.90	21.89	23.27
20	09:03	83.99	14.82	15.07

Table 1



(Figure 4)

DISCUSSION

LAMP is useful primarily as a diagnostic or detection technique, but is not useful for cloning or many other molecular biology applications enabled by PCR. Because LAMP uses 4 (or 6) primers targeting 6 (or 8) regions within a fairly small segment of the genome, and because primer design is subject to numerous constraints, it is difficult to design primer sets for LAMP "by eye".

In a diagnostic application, this must be balanced against the need to choose an appropriate target (e.g., a conserved site in a highly variable viral genome, or a target that is specific for a particular strain of pathogen). Multiple degenerated sequences may be required to cover the different variant strains of the same species. A consequence of having such a cocktail of primers can be non-specific amplification in the late amplification.

Although different mitigation strategies have been proposed for false-positive results in assays based on this method, nonspecific amplification due to various factors including the absence of temperature gating mechanisms is one of the major limitations of Loop-mediated isothermal amplification. We have seen a large number of false positives in our samples.

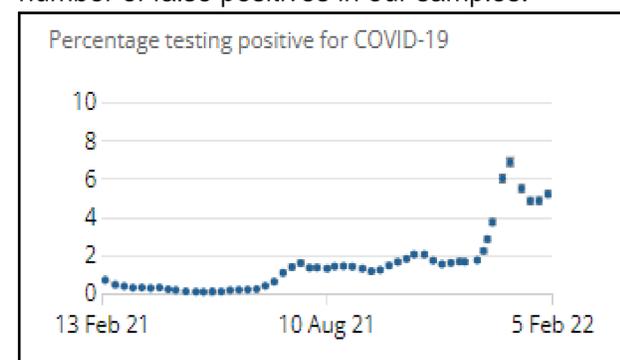


Figure 5 Percentage testing positive for COVID-19 Graph

The positivity rate seen at LAMP testing compared to the national average show similarities in the percentage of positives. From July 5th to the 31st of December we had positive percentage of 0.0157% and then an increase to 0.42% from the 10th of December to the 31st of January, though our positive percentage is a lot lower we were testing asymptomatic staff while the national average tested symptomatic patients.

CONCLUSION

We have implemented a method to routinely test up to 6000 staff every week allowing for close monitoring of patient facing staff who are asymptomatic. The low number of positive results compared to the high volume of samples tested so far shows the great care patient facing staff have taken to limit their exposure to covid by following national guidelines but nonetheless the need for LAMP is clearly outlined as more variants are found and cases continue to rise the monitoring of staff must be maintained as any staff member who may be infected could spread the disease to other staff member and patients potentially causing a outbreak therefore it is best practice to continue testing staff until this matter is resolved

1. Fowler V et al. A highly effective reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of SARS-CoV-2 infection. *J Infect.* 2021 Jan; 82(1): 117–125. Published online 2020 Nov 30. doi: [10.1016/j.jinf.2020.10.039](https://doi.org/10.1016/j.jinf.2020.10.039)

2. Office for national statistics, Percentage testing positive for COVID-19 Graph [Coronavirus \(COVID-19\) latest insights - Office for National Statistics \(ons.gov.uk\)](https://www.gov.uk/government/statistics/coronavirus-covid-19-latest-insights)