Black Country Pathology Services

RT-LAMP; A new method for screening COVID-19 in asymptomatic staff's saliva at the Black Country Pathology Service.

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Introduction

The concerted efforts of NHS staff, military and students have witness to fruition the establishment of the new COVID-19 screening at the Black Country Pathology Service (BCPS) comprising Royal Wolverhampton NHS Trust (RWT), Sandwell and West Birmingham NHS Trust (SWBT), Walsall Healthcare NHS Trust (WHT) and Dudley Group NHS Foundation Trust (DGFT).

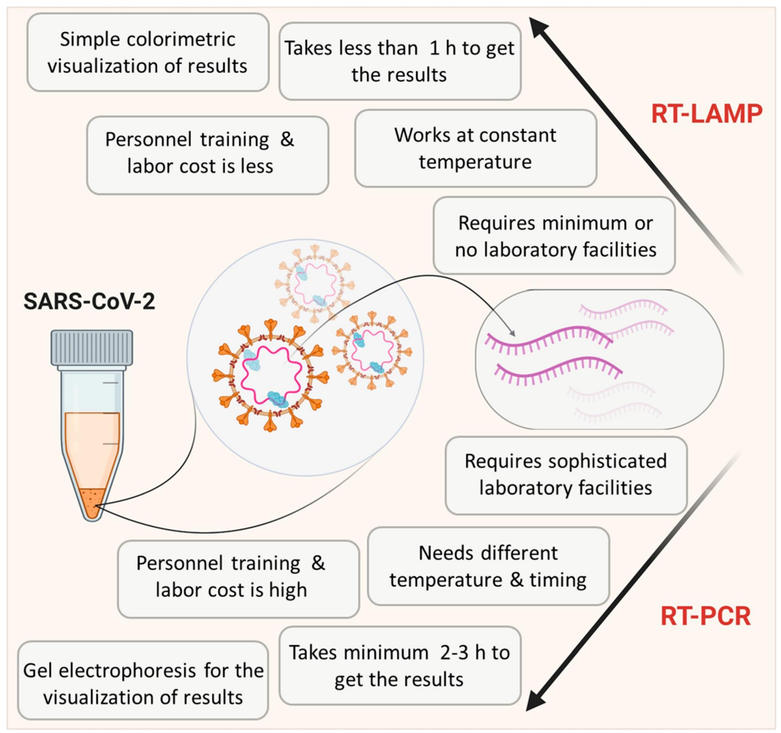

Fig. 1. Schematic comparison of RT-LAMP with RT-PCR (Augustine *et al.*, 2020

Covid 19 has tested the patience of everyone including doctors, scientists and laboratory technicians. Several test regimes has been developed to detect Covid-19 in patient samples. Tests such as PCR and LFD have been largely invasive, time consuming and often expensive, requiring sophisticated transport logistics, skilled personnel to perform and analyse the test result (Fig. 1). As a unique new alternative, RT-LAMP is simple, cheap and inexpensive, providing a point-of-care testing capability.

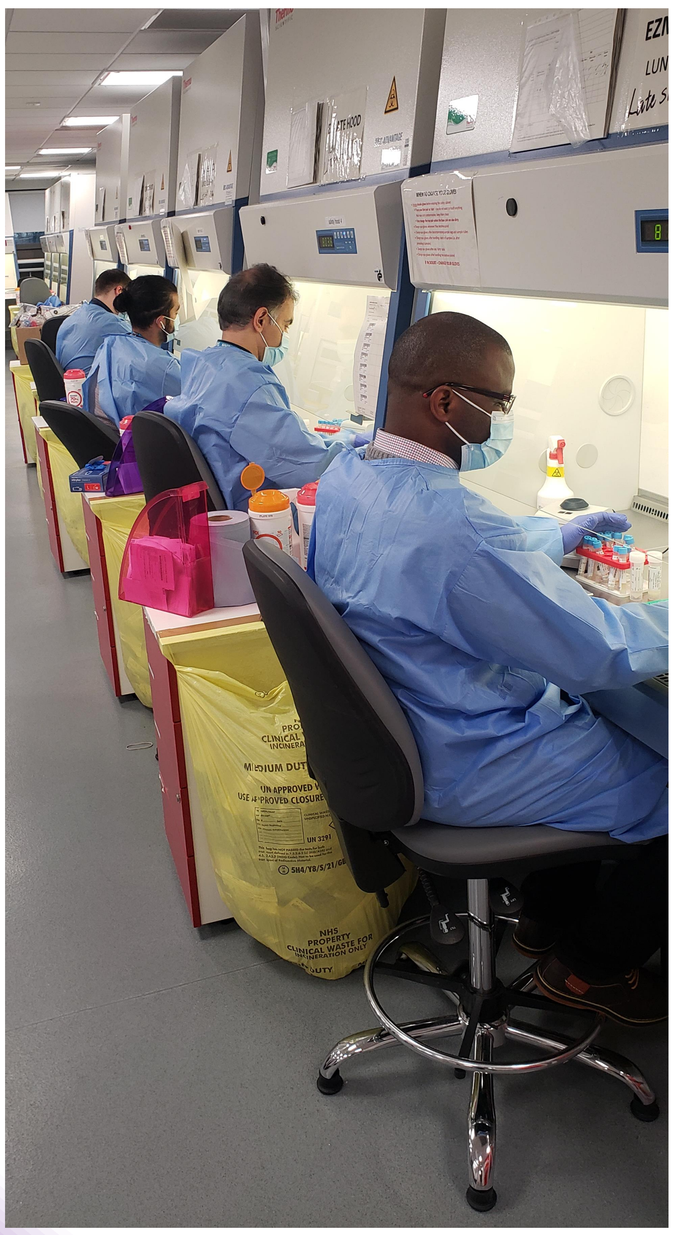

Fig. 5. Pathology (LAMP) Laboratory at RWT

The RT-LAMP *in vitro* diagnostic test is based on the Reverse Transcription Loop-mediated Isothermal AMPlification Technology for the detection of SARS-CoV-2 viral RNA (Fig. 2). The detection is carried out in a one-step, closed tube format where the reverse transcription and subsequent amplification of the specific target sequence occur in a single reaction well.

Method

Asymptomatic staff's weekly saliva samples from the BCPS (190,891) were analysed *in situ* at the RWT laboratory, January to December 2021. The saliva (50µl) was added to the RapiLyse (50µl) and heated to 98°C for 2 minutes in a Themo Fisher Safety Cabinet. An aliquot of 5µl was added to the Reaction Mix; RNA RT-LAMP Master Mix and 10X COVID-19 Primer Mix (17.5 : 2.5) in a Genie well and Placed in Genie HT. The result was then obtained on a digital read-out.

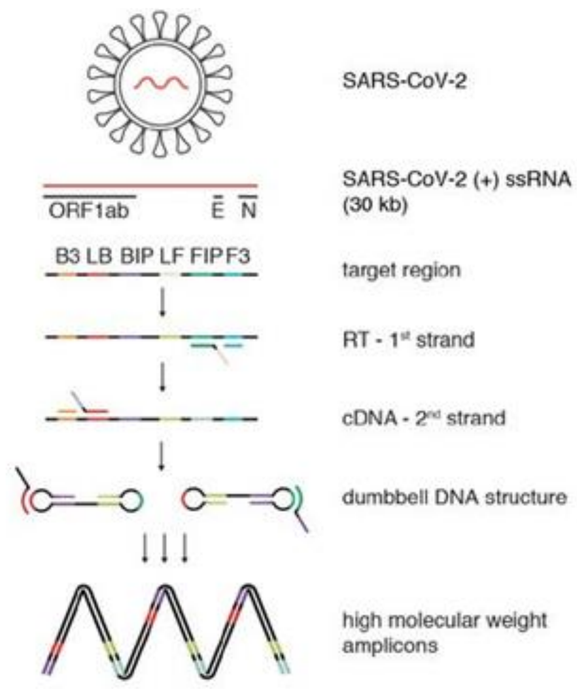

Fig. 2. Schematics of LAMP amplification (Keller *et al.,* 2021)

Result

In all the cases analysed at the BCPS (Fig. 3), there were 1.47 positive cases per 10,000 samples identified with variabilities in their CT, 5.5 – 16.7, and anneal temperature range 83.1°C – 86.5°C. The Genie device detected the amplified product in real-time using fluorescence detection with overall 40 (±1.5) minutes processing time. Test results were subsequently sent out to staff within a 24-hour turn around time (TAT).

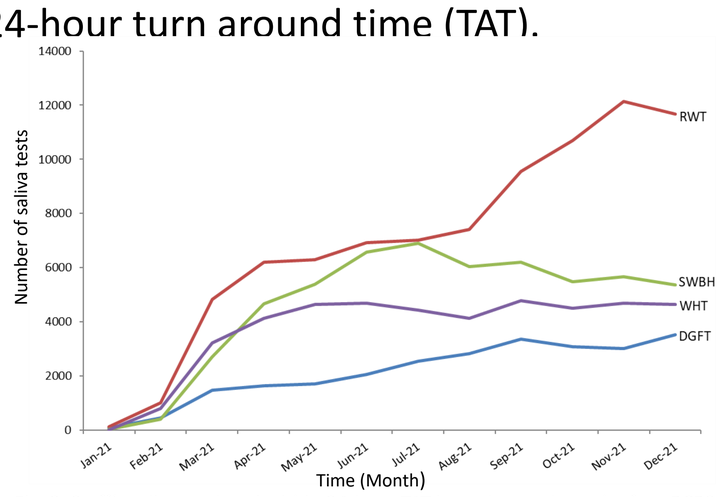

Fig. 3. Staff's saliva analysed within 24-hour TAT in each month on the four BCPS network.

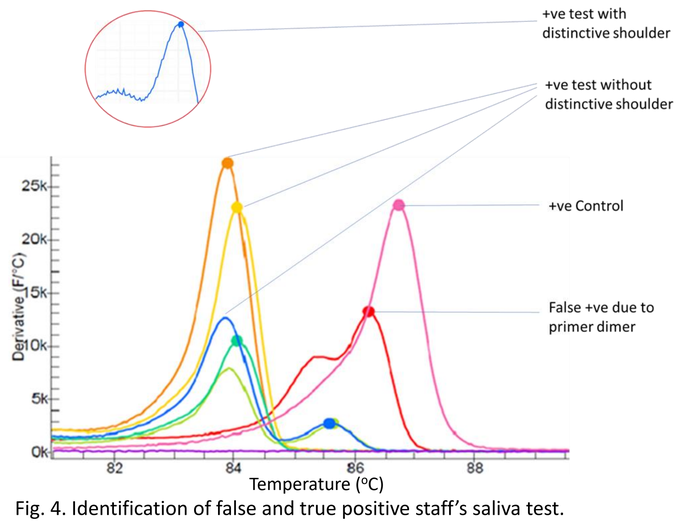

Fig. 4. Identification of false and true positive staff's saliva test.

Discussion

A challenge in data interpretation arose from the variability in analyser results (Fig. 4). Further investigation and repeat testing proved that the true positive test cases occurred with anneal temperature of 83.1 – 83.8°C with a distinctive shoulder while, cases that lied outside this anneal temperature range or without distinctive shoulders are false positive cases which has been ascribed to amplifiable primer dimer complementarity at the base end (Meagher *et al*., 2018). In conclusion, we have successfully implemented the RT-LAMP assay technology for the screening of SARS-CoV-2 viral RNA at BCPS, RWT (Fig. 5). The assay employed a single-tube technique, eliminating the sequential temperature changes and a thermo cycler that resulted in the rapid testing within 24-hour TAT. Following the identification and confirmation of potential infectious positive Covid-19 cases, staff were notified of their test results consequently, breaking infection and reducing transmission within the staff team.

Reference

Augustine, *et al*. Loop-Mediated Isothermal Amplification: A Rapid, Sensitive, Specific, and Cost-Effective Point-of-Care Test for Coronaviruses in the Context of COVID-19 Pandemic. Biology 2020, 9.
Keller, *et al*. A rapid, highly sensitive and open-access SARS-CoV-2 detection assay for laboratory and home testing. Preprint (not certified by peer review) bioRxiv 2021.
Meagher, *et al*. Impact of primer dimers and self-amplifying hairpins on reverse transcription loop-mediated isothermal amplification detection of viral RNA. Analyst. 2018 Apr 16;143(8):1924-1933.