CLARIGENE™ STAYS AHEAD OF THE CURVE WITH RIGOROUS ONGOING VIRAL SURVEILLANCE

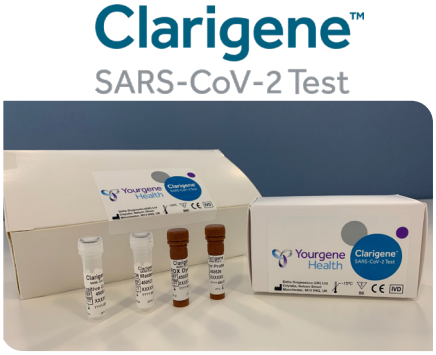
INTRODUCTION

For manufacturers of viral diagnostic assays, close monitoring for the emergence of new strains is essential. This is especially true currently for assays that detect SARS-CoV-2, as the ability to reliably diagnose cases is an important pillar in managing the ongoing pandemic. A viral strain that disrupts a primer or probe site for PCR-based assays could at worst lead to a false negative result.

A virus that encodes its genome in RNA, such as SARS-CoV-2, HIV and influenza, tends to mutate quickly once inside a host primarily because enzymes involved in RNA replication are error prone. However, data suggest that coronaviruses mutate at a slower rate than most other RNA viruses due to an inbuilt proofreading mechanism. On average a SARS-CoV-2 virus mutates at about half the rate of influenza, or at one-quarter of that seen in HIV.

Many of the mutations seen in SARS-CoV-2 have no impact on the ability of the virus to cause or spread disease. Mutations in the 'spike' protein (S) are of particular relevance because of key role of this viral portion in antibody recognition. In late December 2020, the European Centre for Disease Prevention and Control issued a threat assessment brief regarding a SARS-CoV-2 variant with multiple S protein mutations that was in high circulation in the UK (B.1.1.7 lineage, Variant of Concern 202012/01). Analysis at that time suggested this strain was exhibiting increased transmissibility of up to 70%. Since then there have been further lineages for concern detected with multiple spike mutations, such as the 2OH/501Y.V2 variant first identified in South Africa. These developments suggest that, in line with previous experience of viral respiratory illness outbreaks, monitoring of the S gene is of paramount importance.

Developing rigorous surveillance programs that can keep pace with the rapid nature of evolving strains relies on the use of bioinformatic tools, as well as traditional laboratory testing methodologies. Yourgene Health have developed a database monitoring and laboratory workflow to complement the Clarigene™ SARS-CoV-2 assay. This process allows us to be confident of the performance of the Clarigene assay in light of any strains circulating at high levels in the population.



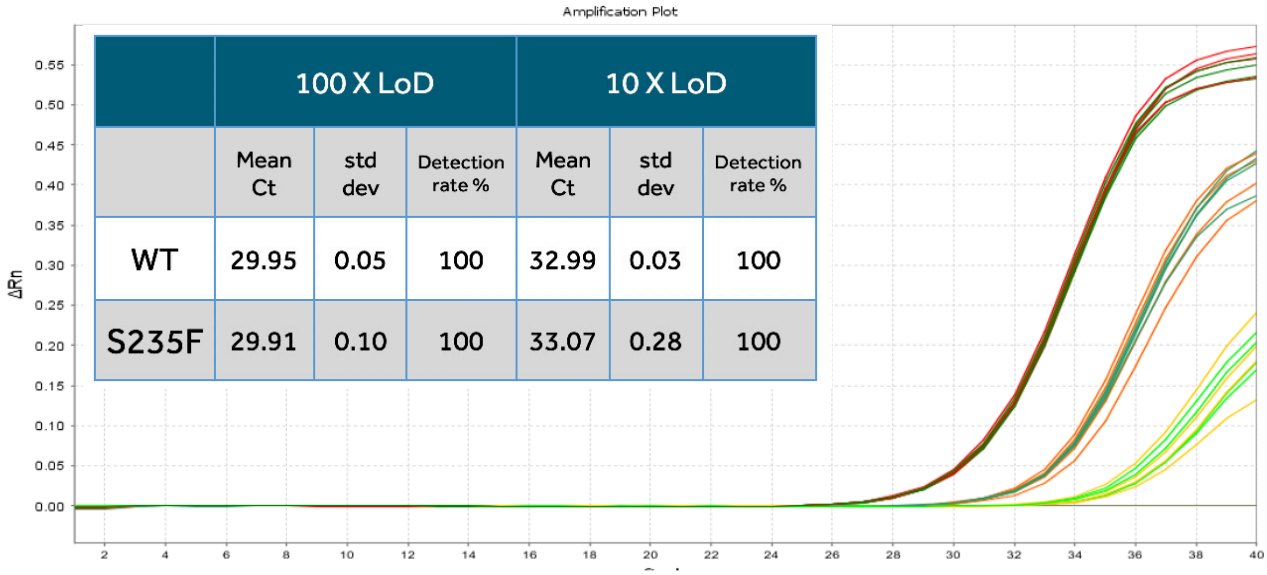
KEY FEATURES AND FINDINGS

- **E and N gene targets.** Unlike many other assays on the market, the Clarigene™ SARS-CoV-2 assay does not rely on amplification of the S gene where it appears viral strain mutations of concern are clustering
- **B.1.1.7 lineage (Variant of Concern 20I/501Y.V1 AKA 202012/01, first observed in the UK).** Performance of the Clarigene assay is not impacted by any currently known mutations (Figure 1)
- **B.1.351 lineage (Variant of Concern 20H/501Y.V2, first identified in South Africa)**. Performance of the Clarigene assay is not impacted by any currently known mutations (Figure 2)
- **P.1 / B.1.1.28 lineage (Variant of Concern 20J/501Y.V3, first identified in Brazilian tourists in Japan).** Performance of the Clarigene assay is not impacted by any currently known mutations

GISAID is a global science initiative and primary source that provides open-access to genomic data of influenza viruses and SARS-CoV-2. Database monitoring using the GISAID initiative as a primary source has so far identified two key viral strands for further investigation through the surveillance pipeline. Table 1 shows mutations present in the B.1.1.7 lineage. *In silico* analysis identified the S235F variant for further follow up with wet lab testing. The results of this wet lab testing can be seen in Figure 1. The same process identified the B.1.351 lineage for investigation, specifically the T205I mutation. The results of this wet lab testing can be seen in Figure 2. *In silico* analysis of the P.1 / B.1.1.28 lineage revealed no variants that would be predicted to impact the performance of the assay and therefore no further testing was undertaken.

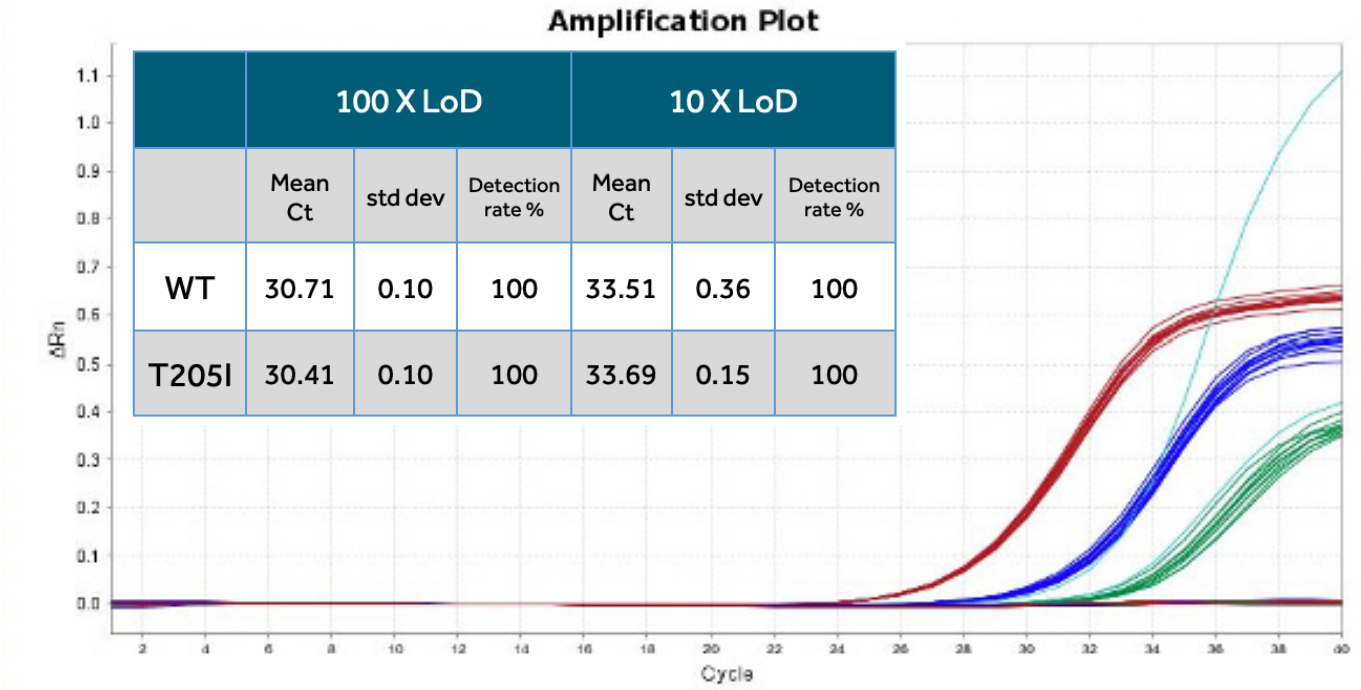
Gene	Mutation
Spike	del 69-70, del 144-145, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
N	R203K, G204R, S235F
E	T9I, L37F, V62F

Table 1. Subset of viral mutations of the B.1.1.7 lineage used for molecular finger printing to interrogate the GISAID database. Clarigene does not rely on amplification of the S gene and as such none of these mutations were identified for further investigation. Of the N&E gene variants only S235F was identified for further follow up based on Clarigene primer positions and the results of previous validation and verification work. Using the same process the N gene mutation T205I was identified for further testing from the B.1.351 lineage (Variant of Concern 2OH/501Y.V2, first identified in South Africa).



	100 X LoD			10 X LoD		
	Mean Ct	std dev	Detection rate %	Mean Ct	std dev	Detection rate %
WT	29.95	0.05	100	32.99	0.03	100
S235F	29.91	0.10	100	33.07	0.28	100

Figure 1. Amplification plot and corresponding mean Ct values for wildtype (WT) and S235F variants of the B.1.1.7 lineage. Testing was performed for WT and mutant S235F samples at 2 different viral load values (100 x LoD and 10 x LoD). The limit of detect (LoD) for the Clarigene assay is 5 copies/reaction. The Ct values illustrate there is no significant difference in performance of the Clarigene assay for WT or S235F samples across arrange of viral loads. Detection rate in both sample types across all viral loads tested was 100%.

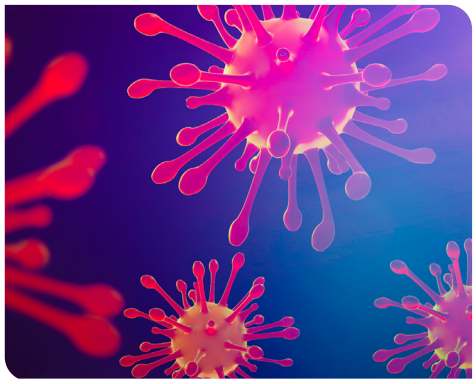


	100 X LoD			10 X LoD		
	Mean Ct	std dev	Detection rate %	Mean Ct	std dev	Detection rate %
WT	30.71	0.10	100	33.51	0.36	100
T205I	30.41	0.10	100	33.69	0.15	100

Figure 2. Amplification plot and corresponding mean Ct values for wildtype (WT) and T205I variants of the B.1.351 lineage (Variant of Concern 2OH/501Y.V2, first identified in South Africa). Testing was performed for WT and mutant T205I samples at 2 different viral load values (100 x LoD and 10x LoD). The limit of detect (LoD) for the Clarigene assay is 5 copies/reaction. The Ct values illustrate there is no significant difference in performance of the Clarigene assay for WT or T205I samples across a range of viral loads. Detection rate in both sample types across all viral loads tested was 100%.

CONCLUSION

Yourgene Health have developed a robust database monitoring and laboratory workflow for viral strain development to complement the Clarigene SARS-CoV-2 assay. This process allows us to be confident that so far the performance of the Clarigene assay is not impacted by any strains circulating at high levels in the population. The design of the Clarigene assay relies not on amplification of the apparently highly mutable S gene, but on the relatively more stable N and E genes. Users can therefore be confident in the ongoing, long-term ability of the assay to detect emerging SARS-CoV-2 strains.



Yourgene Health
Citylabs 1.0
Nelson Street
Manchester
M13 9NQ
United Kingdom

Telephone: +44 (0)161 669 8122
Email: info@yourgene-health.com
www.yourgene-health.com

Your genes. Your health.