Retrospective analysis of data collected on SARS CoV-2 during the pandemic to assess suitability of viral transport media for genomic studies

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Introduction

The COVID-19 pandemic highlighted the growing importance of whole genome sequencing (WGS). The COVID-19 Genomics UK Consortium (COG-UK), a UK-wide collaborative network for SARS-CoV-2 genomics, research and training, was formed to rapidly address the need for sequencing data. To date COG-UK has generated over 2.75 million sequences of the 12.5 million globally, and initially accounted for 25% sequences on the GISAID database[1].

Sequencing has been used for [2]:

- Surveillance and epidemiology of COVID-19.
- Understanding diseases severity of different Variants of Concern (VoC).
- Monitoring the performance of diagnostic assays.
- Assessing vaccine and antiviral therapy
- Modelling to guide UK policy and guidelines.

Sequencing protocols were established using available products remaining from diagnostic testing for COVID-19. At Portsmouth Hospitals University NHS Trust (PHU) this was remaining eluate from COVID-19 PCR positive samples with cT <35.

Samples were nose and throat swabs collected in MWE Sigma Virocult® viral transport media (VTM), extracted on the Qiagen QIASymphony, amplified on the Roche LightCycler 480II, using the Anatolia Geneworks SARS-COV-2 v2 assay. Sequencing was performed on the GridION, Genomics were run by University of Portsmouth Sequencing and Bioinformatics laboratory as part of COG-UK consortium.




Choice of viral transport media is known to be important for PCR and cell culture, with choice of VTM significantly impacting results[3,4]. The role of VTM can vary from enhancing the stability to inactivating the virus [5]. To our knowledge, no studies assessing the suitability of VTM for sequencing currently exist.

The aim of this retrospective analysis is to assess the performance of the methods used and determine if they were suitable for WGS.

Methods

A retrospective analysis of the first 6 months of sequencing data on patient samples (March 2020 – September 2020) was undertaken to assess performance.

The methods used (collection in Sigma Virocult®, extraction on QIASymphony and PCR on the LightCycler480II platforms) were selected as they reflected established and verified methods available for PCR at PHU during this time. Inclusion criteria of a cT <35 was selected for sequencing as cT >35 were likely to reflect very low levels of virus.

Selection criteria for this study:

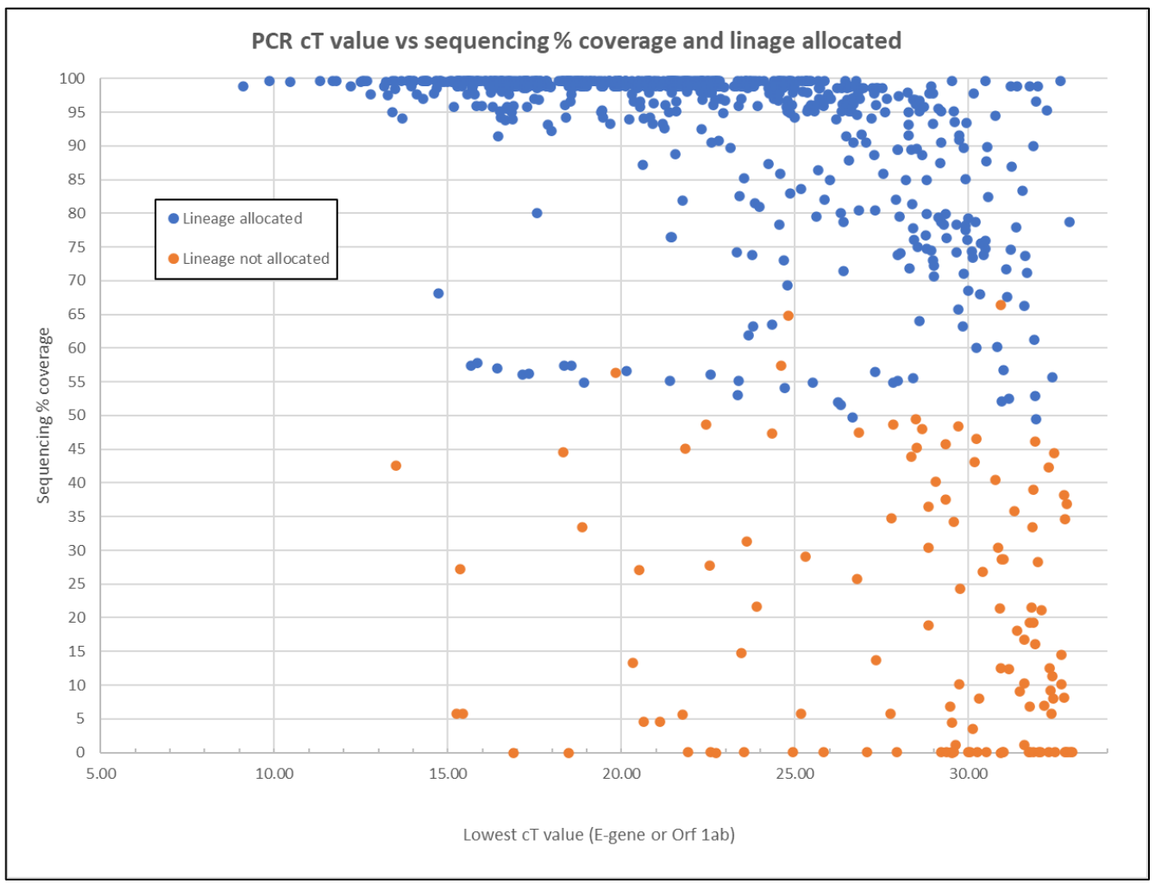
- Only samples which were run through the sequencing platforms are included.
- Only samples which yielded cT values on initial processing are included. (The laboratory implemented Hologic Panther during study period which does not generate a cT value).
- Small portion of data (16 samples) rejected as data for cT values did not compare between Microbiology and Genomics labs. Possibly due to a data capture issue.

Results

791 samples were included in this review. Samples had cT values ranging from 9.1 to 34 for ORF1ab gene, and 9.86 to 32.97 for E-gene. Two samples were only positive in the ORF1ab gene, and 55 samples were only positive in the E-gene, reflecting the higher sensitivity of the E-gene in the assay.

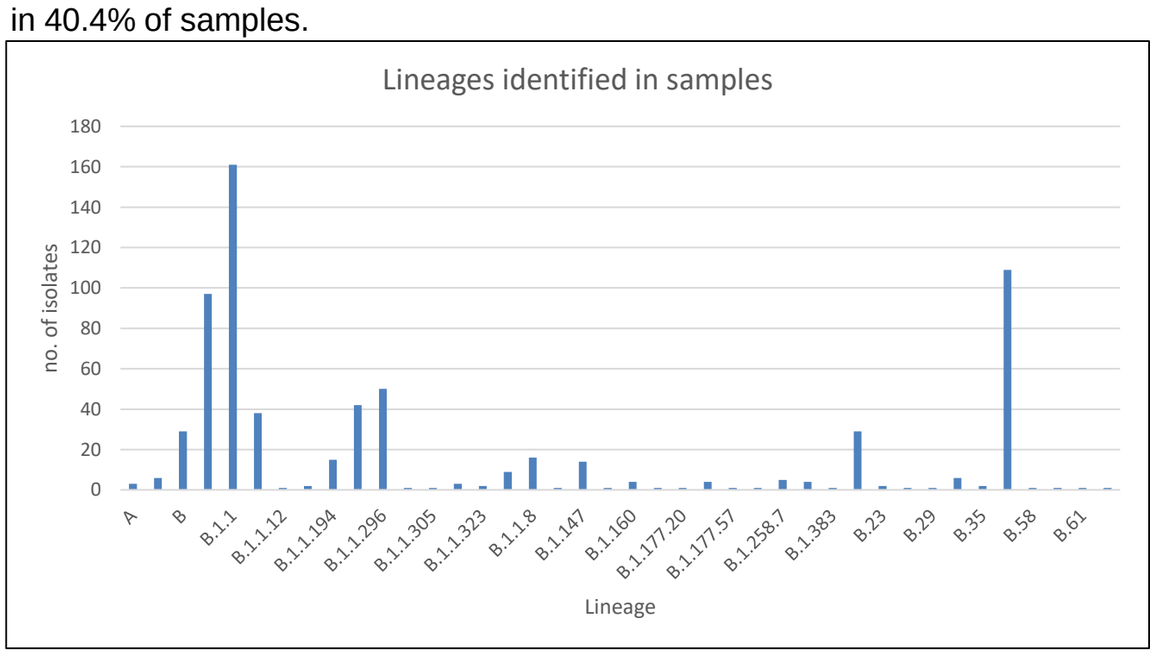
Of 791 samples sequenced, 786 yielded some level of sequencing (0.0033% to 99.5954% coverage), 668 samples yielded enough sequencing to be allocated lineage, **84.5% of all samples referred for sequencing were allocated a lineage**.

Samples where lineage was allocated, sequencing coverage was between 49.52% and 99.60% (PCR cT value range 9.10 - 32.91).
Samples where lineage was not allocated, sequence coverage was between 0.0033% and 66.45% (PCR cT value range 13.50 – 32.97).



Sequencing was successful across the range of cT values. Coverage appear to start declining with cT >27. Lineages were assigned to 94.3% of samples with a cT <=27. Samples where the cT was between 27-34.99 lineage assignment was reduced to 57.9%.

Using the COG UK recommended cut-off of cT <30 a successful lineage was assigned to 91.1% of samples processed. cT >30 but <35 were only successful in 40.4% of samples.



118 samples did not give lineage despite some level of sequencing.
The cT values for these samples ranged from 13.5 – 32.97.

Where coverage was <50% (n=114) lineage could not be assigned. Where coverage was >50 (n=672) lineages were assigned to 99.4% of cases. Four samples without a lineage the coverage range was 56.3 – 66.5%, this is likely a result of incompleteness in the database at that time.

Conclusion

Almost all (789/791) of the positives would have been picked up on E-gene target alone, showing the value of this target in genomic analysis.

cT is a good rough indicator of sequencing success, with the chances of success dropping as cT increases; cT <30 (approximately 1000 cp/ml) are more likely to have successful lineage assignment, with a success rate of 91.1% in this study. However it is possible to get good sequencing results from high cT samples, and also possible to get poor sequencing results from low cT samples.

Approximately 50% coverage is required for lineage to be assigned.

Discussion

To reduce risk of sequencing failure only sequencing sample which are E-gene positive with a cT <30 could be considered.

With the results above we can conclude that the methods used are appropriate for sequencing and Sigma Virocult® from MWE is a suitable media for genomic analysis of SARS CoV-2. This study suggests that Sigma Virocult® is a suitable transport media for RNA viruses, further studies on suitability for genomic analysis of DNA viruses may be indicated.

References

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