RT-PCR genotyping assays to identify SARS-CoV-2 variants in England in 2021: a design and retrospective evaluation study

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Summary

Background Whole-genome sequencing (WGS) is the gold standard diagnostic tool to identify and genetically characterise emerging pathogen mutations (variants), but cost, capacity, and timeliness limit its use when large populations need rapidly assessing. We assessed the potential of genotyping assays to provide accurate and timely variant information at scale by retrospectively examining surveillance for SARS-CoV-2 variants in England between March and September, 2021, when genotyping assays were used widely for variant detection.

Methods We chose a panel of four RT-PCR genotyping assays to detect circulating variants of SARS-COV-2 in England and developed a decision algorithm to assign a probable SARS-CoV-2 variant to samples using the assay results. We extracted surveillance data from the UK Health Security Agency databases for 115 934 SARS-CoV-2-positive samples (March 1–Sept 6, 2021) when variant information was available from both genotyping and WGS. By comparing the genotyping and WGS variant result, we calculated accuracy metrics (ie, sensitivity, specificity, and positive predictive value [PPV]) and the time difference between the sample collection date and the availability of variant information. We assessed the number of samples with a variant assigned from genotyping or WGS, or both, over time.

Findings Genotyping and an initial decision algorithm (April 10–May 11, 2021 data) were accurate for key variant assignment: sensitivities and PPVs were 0·99 (95% CI 0·99–0·99) for the alpha, 1·00 (1·00–1·00) for the beta, and 0·91 (0·80–1·00) for the gamma variants; specificities were 0·97 (0·96–0·98), 1·00 (1·00–1·00), and 1·00 (1·00–1·00), respectively. A subsequent decision algorithm over a longer time period (May 27–Sept 6, 2021 data) remained accurate for key variant assignment: sensitivities were 0·91 (95% CI 0·74–1·00) for the beta, 0·98 (0·98–0·99) for the delta, and 0·93 (0·81–1·00) for the gamma variants; specificities were 1·00 (1·00–1·00), 0·96 (0·96–0·97), and 1·00 (1·00–1·00), respectively; and PPVs were 0·83 (0·62–1·00), 1·00 (1·00–1·00), and 0·78 (0·59–0·97), respectively. Genotyping produced variant information a median of 3 days (IQR 2–4) after the sample collection date, which was faster than with WGS (9 days [8–11]). The flexibility of genotyping enabled a nine-times increase in the quantity of samples tested for variants by this method (from 5000 to 45 000).

Interpretation RT-PCR genotyping assays are suitable for high-throughput variant surveillance and could complement WGS, enabling larger scale testing for known variants and timelier results, with important implications for effective public health responses and disease control globally, especially in settings with low WGS capacity. However, the choice of panels of RT-PCR assays is highly dependent on database information on circulating variants generated by WGS, which could limit the use of genotyping assays when new variants are emerging and spreading rapidly.

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Introduction

During the COVID-19 pandemic, the emergence of SARS-CoV-2 variants was of global importance. Variants of concern (VOCs) changed the transmission, hospitalisation, or mortality[1] associated with SARS-CoV-2 infection, and were consequently globally important for public health. VOCs substantially affected the COVID-19 pandemic, starting with the emergence of the alpha VOC (B.1.1.7) detected in England in November, 2020,[2] with other VOCs subsequently emerging.[3] Further detail of the epidemiology of SARS-CoV-2 variants is provided elsewhere.[4]

Whole-genome sequencing (WGS) was crucial to identify emerging variants in the population.[5] WGS also had a key role in large-scale transmission dynamic studies and in understanding pathogenesis and immune response in individuals infected with SARS-CoV-2.[6] However, for large-scale monitoring of variants, WGS has technical, logistical, and financial limitations. Capacity for WGS varies globally, and within most countries only a small proportion of COVID-19 cases were sequenced.[5] Additionally, turnaround time from sample collection to sharing of variant information with WGS can take 1–2 weeks.[7] In a rapidly

Research in context

Evidence before this study

Within infectious disease epidemiology, RT-PCR genotyping assays are used to detect small genetic differences in organisms. These differences might be key to understanding disease transmission, antimicrobial resistance, changing disease infectivity, or severity of clinical illness. Such information is essential for disease surveillance enabling public health action. Whole-genome sequencing (WGS) is the gold standard diagnostic tool to identify and genetically characterise variants, but in circumstances in which very large populations need to be assessed rapidly, cost, capacity, and timeliness limit its usefulness. We searched Scopus without language restrictions for articles published between database inception and Oct 10, 2022, searching for article titles, abstracts, or keywords containing ("rt-pcr" or "genotyping" or "assay") AND "disease surveillance". We found that the potential of genotyping assays for high-throughput mass variant surveillance is under-investigated. Existing studies frequently reported on very small throughput systems, simply discussed the development of the assays, or were focused on specific subgroups of the population.

Added value of this study

This research focused on a high-throughput system for SARS-CoV-2 surveillance, where at its peak around 50 000 samples per week were genotyped with RT-PCR assays. We used the assay results to assign the probable SARS-CoV-2 variant and have shown that for most variants specificity was high. We have shown that genotyping produced variant information for public health action a median of 3 days after the patient sample date, which was faster than WGS (median 9 days). We have also shown that genotyping was cheaper, and the flexibility of the process enabled a nine-times increase in the quantity of samples tested for variants.

Implications of all the available evidence

We highlight that RT-PCR genotyping assays can be suitable for high-throughput surveillance of variants of pathogens such as SARS-CoV-2, as a complement to WGS, particularly when variants are not rapidly changing. Through an analysis of SARS-CoV-2 variants circulating in England in 2021, RT-PCR genotyping assays had a high degree of accuracy and, compared with WGS, were less resource intensive, time from sample collection to result availability was reduced, and had a greater flexibility to change capacity. Greater speed and flexibility are linked to the relative low cost of genotyping assays and the ease with which they can be implemented in laboratories using existing equipment and staff training. RT-PCR genotyping assays provide important potential for guiding public health decision making and disease control globally.

changing epidemiological situation (eg, with cases of the delta VOC [B.1.617.2] doubling every 4·5 days[8]), WGS is of less use for a rapid public health response, such as contact tracing or local travel restrictions.[9]

For more information on **standard case definitions**, see https://github.com/phe-genomics/variant_definitions

The genomes of SARS-CoV-2 variants carry a characteristic set of mutations that can be detected using real-time RT-PCR genotyping assays, which can be used as an alternative to WGS. These assays are designed to differentiate between mutant and reference sequences at a specific position. Variant mutations can lead to failure of amplification of targets in some diagnostic COVID-19 assays, indicating the presence of a variant in the sample. Genotyping can complement WGS by offering increased scalability, reduced financial cost, and increased speed of result.[10]

Within England, microbiology and virology National Health Service (NHS; public) and private laboratories undertook PCR testing for SARS-CoV-2. Community and wider-population home-based testing was processed in high-throughput Lighthouse Laboratories with the TaqPathCOVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA), which targets the S, N, and Orf1ab genes of SARS-CoV-2. All PCR testing results were reported to the UK Health Security Agency (UKHSA) Second Generation Surveillance System (SGSS).[11] As part of this testing, RNA extracted from samples collected from specific groups or categories of individuals (eg, international travellers) and 10–15% of community PCR-positive samples were sent for WGS. The Wellcome Sanger Institute (Hinxton, UK) undertook most WGS from March, 2021, and all results were input into the national Cloud Infrastructure for Big Data Microbial Bioinformatics (CLIMB) database. Subsequently, the UKHSA assigned a variant to each sample using standard case definitions. Case-level information, including variant information, was disseminated to local health protection teams for epidemiological investigation and public health action. All testing was underpinned by comprehensive quality assurance processes.[12]

Coinciding with the emergence of the alpha VOC and to overcome some of the limitations of WGS, in February, 2021, laboratories started conducting secondary tests on positive PCR samples using panels of RT-PCR genotyping assays[7] to detect a range of variants. These results were also reported to the SGSS.

We aimed to assess the potential of genotyping assays to provide accurate and timely variant information at scale by retrospectively examining surveillance for SARS-CoV-2 variants in England between March and September, 2021.

Methods

Study design

In this retrospective analysis, we focused on the period from March 1 to Sept 6, 2021, when genotyping assays were introduced and widely used for variant assignment within England. Over this period, cases fluctuated between 3000 and 25 000 per day.[13] Using a decision algorithm to determine probable variant, we subsequently retrospectively evaluated the utility of RT-PCR genotyping within a national surveillance strategy for monitoring variants. We

focused on their accuracy (sensitivity, specificity, and positive predictive value [PPV]), and benefits such as speed of result, cost, and increased capacity for testing compared with WGS.

Ethics approval was not required for this study because it was part of routine care and surveillance in England. Data were collected for contact tracing and health protection purposes, falling under Regulation 3 of the Health Service (Control of Patient Information) Regulations 2002.

Procedures

Genotyping assay target selection

In February, 2021, we assessed a number of mutations for inclusion in a panel of genotyping assays to determine whether they could detect circulating variants considered to be of greatest concern to public health (alpha, beta [B.1.351], gamma [P.1], and any with E484K[14]). We excluded mutations that were common to multiple VOCs or those prone to WGS issues. Following a reassessment of circulating variants in England in April, 2021, we repeated the selection to allow detection of emerging delta VOC.[15] From February, 2021, laboratories started undertaking secondary tests on positive PCR samples using the initially approved genotyping assays. After April 15, 2021, laboratories changed the assays used to enable differentiation of the emerging delta VOC.

Genotyping assay

For assay targets, we used the TaqMan SARS-CoV-2 mutation panel (Thermo Fisher Scientific, Waltham, MA, USA). The assays were used with TaqPath 1-Step RT-qPCR Master Mix, CG (Thermo Fisher Scientific) with remaining RNA extracted for the initial diagnostic detection of SARS-CoV-2. Two TaqMan minor groove binding probes with non-fluorescent quenchers were included in each assay: one probe for detection of the wild-type sequence (VIC labelled) and another probe specific for mutated sequence (FAM labelled).

Development of a decision algorithm

On April 9, 2021, we extracted genotyping assay, S-gene target surveillance (SGTS), and WGS variant results from all 2674 samples with this information from the CLIMB and SGSS databases. We categorised each of the four genotyping assays and the SGTS result (henceforth genotyping) into one of four requirements statements: mutation is present, mutation is not confirmed absent, mutation is present or absent, or mutation is not confirmed present (appendix p 1). The statements categorised as not confirmed present and not confirmed absent accommodated missing or inconclusive results. All combinations of these requirements statements were identified and cross referenced to the variant derived from WGS. We proposed a decision algorithm to derive a probable variant on the basis of the specific combination of genotyping results in the sample using our own knowledge of variant mutations. Through an iterative process of proposing decision algorithms and calculating their accuracy against the samples with genotyping and WGS results, we developed a final decision algorithm. An amended version was developed at the start of May, 2021, because of the new assays used in laboratories.

Statistical analysis

The routine use of a decision algorithm to monitor probable sample variant on the basis of results from genotyping assays within a working surveillance system allowed us to evaluate genotyping. For every sample for which there was a variant from both the genotyping assay panel and WGS (paired variant results), we generated a cross-tabulation comparing the variant assigned to samples from the genotyping assay (using the decision algorithm) to that from WGS. We calculated three measures of variant assignment accuracy and their associated 95% CIs: sensitivity of the genotyping panel (proportion of samples with a specific variant identified by WGS correctly classified by the decision algorithm), specificity (proportion of samples not classified as the variant by WGS, that were classified as such by the decision algorithm), and PPV (proportion of samples for which the decision algorithm classification was confirmed by WGS). Variant assignment accuracy was calculated for all samples together, and was assessed every week to evaluate changes over time. The accuracy metrics we used are fully described elsewhere.[16]

To assess timeliness of variant surveillance, for every sample for which there was a variant from both genotyping and WGS (paired variant results) we calculated the time between sample date (specimen date) of the PCR-positive sample and both the availability of genotyping and WGS result. The lag time between the two methods was also calculated on a per-sample basis. These data were summarised as mean (SD) and median (IQR) time lags. All paired variant results were analysed between March 1, 2021, and Sept 6, 2021. Data were subdivided by week to explore changes in timeliness.

We assessed the increase in national capacity to assign variants due to genotyping assays by extracting all PCR-positive samples identified between March 1 and Sept 6, 2021. We identified the number of samples for which variant information came from genotyping only, WGS only, or both genotyping and WGS. Data were subdivided by weeks to explore changes over time.

All statistical analysis was done in R (version 4.2.1).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

See Online for appendix

Results

For assigning probable variants to SARS-CoV-2-positive specimens, four assay targets plus SGTS were chosen and approved by the UKHSA Variant Technical Group. These were N501Y, E484K, K417N, and K417T of the TaqMan SARS-CoV-2 mutation panel.

The assay target N501Y affects a contact residue within the receptor binding domain of the spike protein and increases

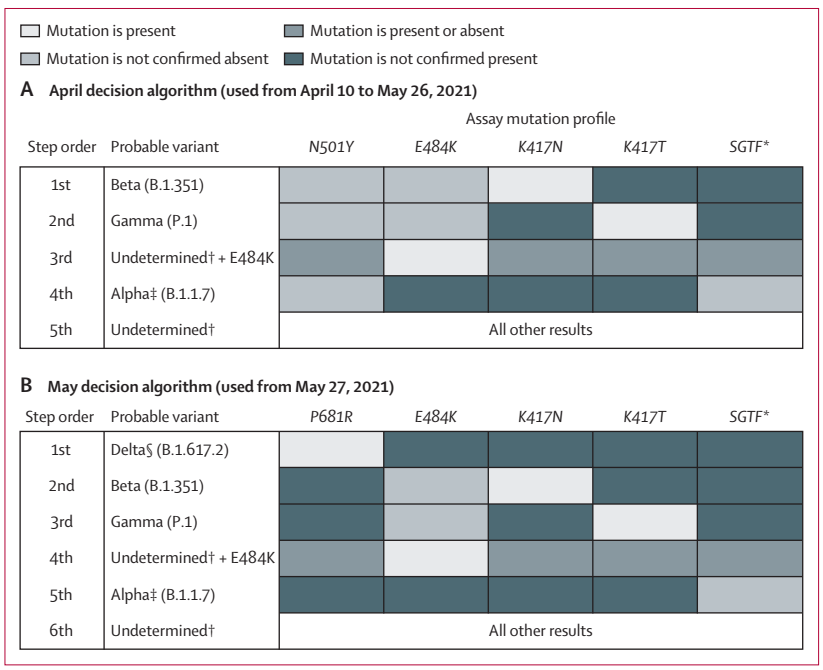

Figure 1: **Decision algorithms used to assign a probable variant from genotyping assay results**
Evaluation of variant information derived from genotyping assays in routine surveillance. *Proxy for del69_70. †Samples with an assay result but for which a pattern of assay gene target results do not identify one of the currently called variant profiles. ‡Mutation must be present in either N501Y or SGTF to meet definition for alpha. §Genotyping assay with P681R target could not distinguish B.1.617.1, B.1.617.2, and B.1.617.3 lineages and is used as a proxy for B.1.617.2, given contemporary prevalence of this lineage.

resistance to neutralising antibodies.[17] E484K confers decreased sensitivity to convalescent and vaccine-induced immune sera and resistance to monoclonal antibody therapies.[17,18] K417N and K417T have similar properties to E484K and are mutations found in the beta and gamma VOCs.[17,18] In May, 2021, N501Y was replaced with P681R in the assay panel to account for increase in the delta variant. P681R is characteristic of the delta variant and enhances viral fusion.[19] It was recognised that this change would limit ability of the genotyping assay to differentiate alpha, but by April, 2021, this variant was of lower public health importance.

The first decision algorithm (April decision algorithm) was implemented in routine surveillance from April 10, 2021. From May 27, 2021, an amended decision algorithm was developed (May decision algorithm) and implemented in routine surveillance.

Figure 1 shows the two decision algorithms. Each consisted of five or six sequential steps (step order) to assign a probable variant from the genotyping assay results. This step order partly reflects the public health importance of different variants. For example, using the April decision algorithm sample, genotyping results were first checked against the beta profile. If the sample did not meet this definition, results were then checked against the gamma mutation profile, and so on.

The table shows the paired comparison between the variant derived from the genotyping panel (April decision algorithm) and the WGS data from April 10 to May 11, 2021. From genotyping, the alpha variant predominated (84·6%, 13 042/15 424); the accuracy of identification of the alpha variant by genotyping was high, with sensitivities (0·99, 95% CI 0·99–0·99), specificities (0·97, 0·96–0·98), and PPV (0·99, 0·99–1·00) all greater than 0·95 (table). During this period, weekly sensitivity and PPV were consistently high for the alpha variant. Due to the dominance of this variant, at the start of the period there were very few true negatives, resulting in a lower specificity (appendix p 2). As new variants emerged the number of true negatives increased, leading to an increase in specificity. The beta (0·5%, 81/15 424) and gamma (0·1%, 23/15 424) variants both showed high sensitivities, specificities, and PPVs (table). The small number of samples mean that the 95% CIs around the accuracy metrics for the gamma variant are large (table). The remaining samples were classified as undetermined, most of which were confirmed as the delta variant by WGS (a variant that the April decision algorithm was not designed to differentiate).

The table shows the performance of the amended decision algorithm, covering May 27 to Sept 6, 2021. Within the genotyping-derived variant, the delta variant predominated (95·6%, 96 062/100 510); sensitivity (0·98, 95% CI 0·98–0·99), specificity (0·96, 0·96–0·97), and PPV (1·00, 1·00–1·00) of genotyping assays for identification of the delta variant were all greater than 0·95 (table). During this period, weekly sensitivity and PPV were consistently high for the delta variant (appendix p 2). Due to the increasing dominance of the delta variant throughout this period, weekly specificity initially started high and then decreased as the number of true negatives decreased to zero. Small numbers of beta (0·01%, 12/100 510) and gamma (0·02%, 18/100 510) variants were identified with high accuracy but large 95% CIs around the metrics (table). Removal of the N501Y target reduced sensitivity of the assay panel to the alpha variant, and many of these cases were subsequently called as unclassified. This finding is unsurprising since none of the mutation assays were specific for the alpha variant, and identification relied on SGTF only.

Figure 2 shows the median time between sample collection date and availability of a variant profile from a genotyping or WGS approach. Median time between sample date and a variant determination was 3 days (IQR 2–4; mean 4·0 days, SD 6·8) with genotyping assays, and 9 days (IQR 8–11; mean 10·1 days, SD 4·4) with WGS. Genotyping assay results were available a median of 6 days (IQR 5–7; mean 6·1, SD 7·7) faster than were results with WGS. Figure 3 shows how this advantage changed over time because of operational process improvements. In March, 2021, the median time between sample date and availability of a variant from WGS was 21 days (IQR 19–23), reducing to 9 days (8–9) from early May, 2021. The time to a variant result from genotyping assay remained relatively constant at 3 days. Figure 3 also shows that the surge in samples processed around June, 2021 (peak in weekly total positive COVID-19 samples; June, 2021, peak of the delta variant) had little effect on median turnaround.

	WGS variant								Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)
	Alpha*	Beta	Gamma	Unclassified† + E484K	Undetermined‡	Delta§	Other¶	Total			
April decision algorithm, using surveillance data from April 10 to May 11, 2021											
Alpha	12 970	0	2	0	28	26	16	13 042	0·99 (0·99–0·99)	0·97 (0·96–0·98)	0·99 (0·99–1·00)
Beta	0	81	0	0	0	0	0	81	1·00 (1·00–1·00)	1·00 (1·00–1·00)	1·00 (1·00–1·00)
Gamma	1	0	21	1	0	0	0	23	0·91 (0·80–1·00)	1·00 (1·00–1·00)	0·91 (0·80–1·00)
Undetermined‖ + E484K	13	0	0	1	0	0	102	116	..	..	..
Undetermined‖	116	0	0	0	40	1931	75	2162	..	..	..
Grand total	13 100	81	23	2	68	1957	193	15 424	..	..	..
May decision algorithm, using surveillance data from May 27 to Sept 6, 2021											
Alpha	1004	0	0	0	0	5	8	1017	0·35 (0·33–0·37)	1·00 (1·00–1·00)	0·99 0·98–1·00)
Beta**	0	10	0	0	0	0	2	12	0·91 (0·74–1·00)	1·00 (1·00–1·00)	0·83 (0·62–1·00)
Gamma	1	0	14	0	0	3	0	18	0·93 (0·81–1·00)	1·00 (1·00–1·00)	0·78 (0·59–0·97)
Undetermined§ + E484K	7	0	1	0	0	1	44	53	..	..	..
Undetermined§	1829	1	0	0	14	1498	6	3348	..	..	..
Delta††	43	0	0	0	58	95 951	10	96 062	0·98 (0·98–0·99)	0·96 (0·96–0·97)	1·00 (1·00–1·00)
Grand total	2884	11	15	0	72	97 458	70	100 510	..	..	..

WGS=whole-genome sequencing. PPV=positive predictive value. *Includes alpha and alpha with E484K. †Unclassified (no match to standard definitions). ‡Unclassified and undetermined (low CT values). §For the April decision algorithm, includes delta and delta with K417N; for the May decision algorithm, includes delta, delta with E484K, delta with K417N, and the AY4.2 delta subvariant. ¶For the April decision algorithm, includes the eta (B.1.525), B.1.1.318, B.1.617.3, AV.1, C.36.3, and kappa (B.1.617) variants; for the May decision algorithm, includes the kappa, B.1.1.318, AV.1, and C.36.3 variants. ||Samples with an assay result but for which a pattern of assay gene target results does not identify one of the currently called variant profiles. **Includes two cases of mu (B.1.621), which were indistinguishable from beta using the May decision algorithm. ††The May decision algorithm can differentiate between delta and delta + E484K and delta + K417N, but these variants are combined here.

Table: **Concordance of matched genotyping assay and WGS variant results with use of the April decision algorithm and May decision algorithm**

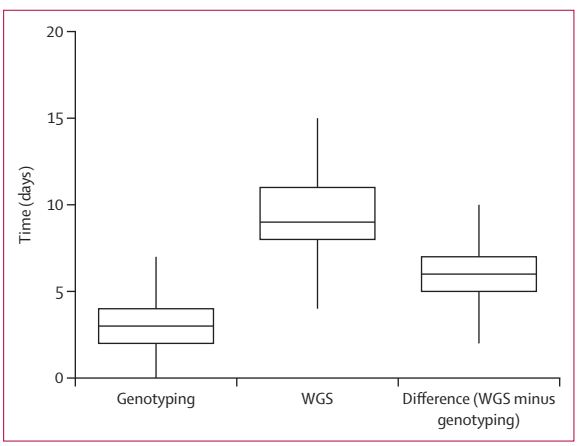

Figure 2: **Boxplots of the time between the sample date of a positive case and the availability of a variant derived from genotyping assays, WGS, and the difference between these two times (March 1–Sept 6, 2021)**
Whiskers show ± 1·5 × IQR. The difference between these two times was calculated on an individual sample basis. WGS=whole-genome sequencing.

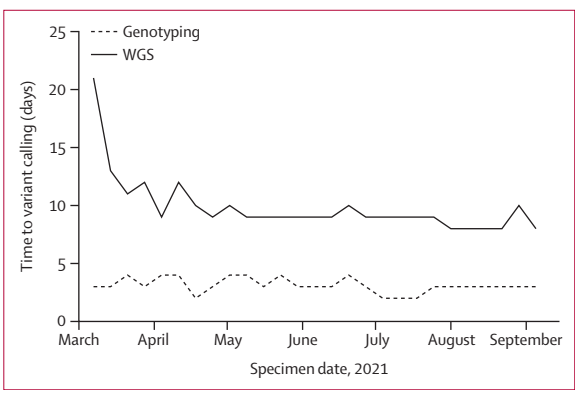

Figure 3: **Median time between the sample collection date of a positive sample and the availability of a probable variant from the panel of genotyping assays and the variant from WGS pipelines (March 1–Sept 6, 2021)**
WGS=whole-genome sequencing.

Figure 4 plots weekly number of samples with variant assigned from genotyping assays and from WGS, and shows total weekly positive COVID-19 samples. Results indicate that the total number of COVID-19-positive samples with variant information dropped initially, then started rising from early May, 2021. Until early April, 2021, most variant information was provided by WGS. Between mid-May and mid-June, 2021, cases with a variant assigned from genotyping increased from 5000 to 45 000 per week, providing important public health information during early phases of the delta variant. During this same period, samples with a variant assigned from WGS increased from 5000 to 20 000 per week, which was the national WGS capacity. From mid-July onwards, the number of samples with a variant assigned from WGS increased to 30 000 (new national WGS capacity) while the number of samples with a variant assigned from genotyping assays reduced, with a dip in mid-July. This dip was due to a spike in primary PCR testing demand (figure 4), resulting in resources from the

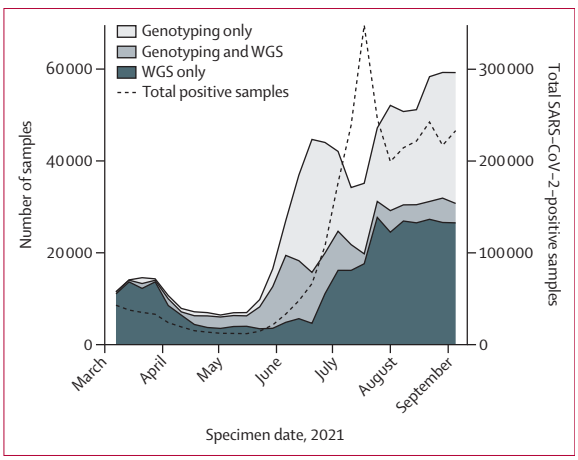

Figure 4: **Number of VOCs reported to SGSS with a genotyping result, a WGS variant result, or both, by week (March 1–Sept 6, 2021)**
VOC=variant of concern. SGSS=Second Generation Surveillance System. WGS=whole-genome sequencing.

genotyping assay processing being redirected to maintain capacity and turnaround times for primary PCR testing. Turnaround times for samples that were genotyped remained constant.

Discussion

In this study we have shown how RT-PCR genotyping panels, underpinned by comprehensive quality assurance processes for all testing, can complement WGS by providing accurate, rapid, and scalable methods for assignment of known variants.

Effective public health action is driven by surveillance, the objectives of which are to describe the burden and epidemiology of disease, monitor trends, and identify outbreaks and novel pathogens. From April, 2021, genotyping results were incorporated into the daily line list of SARS-CoV-2 variant cases in England. RT-PCR allowed timely and improved characterisation of transmission patterns and risk factors to inform public health action and policy, including travel restrictions and the timing of vaccination delivery programmes. During the emergence of new variants, variant information was rapidly communicated (within 24 h) to front-line health protection professionals who were responsible for implementing local control measures. Genotyping allowed these teams to link cases to each other and to specific premises so that prompt public health action (eg, contact tracing) could be taken to prevent further transmission. However, the usefulness of individual public health measures that focused on VOCs is limited because at no point were all cases assigned a variant. Maximising the benefits of a genotyping approach depends on effectively prioritising which samples would benefit most from variant assignment (eg, samples from individuals reporting recent foreign travel). Additionally, genotyping was used to rapidly highlight the importance of travel cases causing importation and subsequent dominance of the delta variant.

The genotyping approach that we describe was based on five assays (including SGTF) and produced results that were deemed sufficiently accurate. In any surveillance system incorporating genotyping panels, the number of assays is crucial, to balance technical and cost considerations with ability to assign probable variant.[20] This balance depends on the number of variants, characteristics or defining mutations in their viral genome, and resourcing given that each assay has additional cost. Each assay also uses some sample RNA, which might negatively affect the amount of RNA left for other purposes (eg, WGS) in small volume samples. During the omicron variant, there was a large increase in recombinant lineages whereby two different lineages infected the same cell (eg, Omicron XE recombinant BA.1 x BA.2).[21] Genotyping was phased out from March, 2022, coinciding with the emergence of the omicron variant and reduction in community testing. Had genotyping continued during the omicron phase, distinguishing recombinant lineages would have required additional targets within the genotyping assay with associated additional costs.

We have shown the timeliness of the genotyping approach. Probable variant assignment from genotyping assays were reported a median of 6 days before results from WGS. Shorter time taken to report a variant from genotyping assays was due to tests being done at the initial diagnostic laboratory as a pre-planned measure, whereas WGS involved transfer of samples to a centralised facility. WGS also requires enhanced sample preparation and longer analytical procedures.[10] In the future, rapid sequencing approaches might reduce this time,[22] but scalability of these approaches is unclear. In a rapidly changing epidemiological situation the time advantages of genotyping assays are notable. In our study period, cases of the delta variant were doubling every 4·5 days in some regions.[8] Rapid identification of variants through genotyping and the larger number of samples tested than with WGS allows for more timely assessment of transmissibility, infectivity, and severity of emerging variants. Genotyping assays provided rapid information to guide the potential for differential management of cases according to variant, and for variant-specific disease modelling.

Related to timeliness is the ability to develop and roll out new genotyping assays in response to emerging variants. The timescales between the emergence of a new variant, WGS data becoming available, development and roll-out of new genotyping assay panel, and incorporation of these data into decision making are key to its usefulness. We have shown that during the emergence of the delta variant this process occurred within 3–4 weeks, which is relatively fast from a laboratory perspective. This timeliness was because laboratories had prepared processes and held samples in anticipation of this need, and because of rapid vendor response to provide new primers alongside UKHSA infrastructure to quickly generate quality control materials. Thus continuously monitoring and adjusting genotyping processes can allow for its continuous use in surveillance even when variants change. However, genotyping during the

omicron variant phase would have presented challenges owing to its rapid growth. The omicron VOC (lineages BA.1, BA.2, BA.4, and BA.5) emerged and grew rapidly to dominance in the population, all within 5 months.[23] Although the development and roll-out of new genotyping assay panel occurred within days of the emergence of the new omicron variant, it was a month from emergence to the genotyping results being incorporated into decision making. By this point half of all cases were attributed to the omicron variant, making a genotyping assay approach of little use[24] for BA.1 and subsequent omicron variants.

Despite genotyping assays only being implemented in April, 2021, by mid-June there were 45 000 samples tested each week using a genotyping approach. The nine-times increase (5000–45 000 samples per week) in number of samples tested for a variant using a genotyping approach shows an ability to rapidly scale capacity in response to emerging trends. Genotyping assays can be speedily deployed because of the low cost of assays and the ease with which they can be implemented in laboratories using existing equipment and staff training. WGS requires expensive equipment and highly specialised labour that are available only at a few centralised facilities, leading to fixed capacity constraints. The distributed nature of testing for variants using genotyping has additional benefits as back-up for when issues with WGS arise at centralised facilities. In mid-June, 2021, an operational issue at a WGS site affected around 20 000 samples, causing over a quarter of sample results to be of insufficient quality for variant detection. During this period, variants were monitored using results from genotyping assays, which affected variant assignment, acquisition of epidemiological intelligence on variants, and public health action.[25] The usefulness of a genotyping assay approach must be set against the observation that variant assignment is only probable (although accuracy metrics are high), and additional mutations within the variant cannot be identified. Additionally, other than probable variant, the relatedness of samples cannot be explored, making it challenging, for example, to identify individual outbreaks.[26]

Several factors affect sample costs including throughput, vendor, and setting, but during the study period the per sample cost to the UKHSA of genotyping varied between £10 and £30, whereas WGS varied between £30 and £55. A small US study reports similar costs for genotyping (£15) but a much higher WGS cost (£130).[27]

Recent surveys have highlighted that the UK is one of the nations with the highest WGS capacity, and reports WGS information in a timely manner.[5,7] Hence, the timeliness, cost, and capacity advantages of genotyping assays that we report for the UK might be greater in other settings. However, the design of genotyping assays depends on WGS-generated database information on variants in circulation. Furthermore, time from the identification of a new variant to WGS and the subsequent genotyping assay results being incorporated into decision making can take weeks. This delay could limit the use of genotyping assays when new variants are emerging and rapidly spreading. Genotyping assays can be applied to the assignment of mutations in a wide range of organisms such as other viruses, bacteria, and protozoa,[28,29] in humans and non-humans.[27] Genotyping assays can also be used in environmental sampling.[30] The potential benefits of genotyping assays shown in this paper in terms of accuracy, cost, timeliness, and ability to rapidly change capacity extend far beyond SARS-CoV-2. They provide important potential for guiding public health decision making and disease control globally.

Contributors

NB, WS, ME, RE, and IRL conceived of the study. AD envisioned the use and oversaw the implementation of genotyping within Lighthouse Laboratories across the UK with support from KR. AD, MJW, HV, MLS, EG, PJ, and ME developed and interpreted the assay panel data. NB did the statistical comparison of genotyping and WGS data, with data extraction and analytical support from AMOC, EG, WS, IRL, PB, and RE. NB, IRL, WS, and RE led on paper writing, with support and critical revisions received from AMOC, EG, ME, MJW, HV, JDMF, PJ, MLS, PB, DH, RV, KR, AD, and SH. All authors have read and agree to the published version of the manuscript. All UKHSA authors had full access to all the data in the study and all authors accept responsibility for the decision to submit for publication. NB and EG verified underlying data of the study.

Declaration of interests

JDMF and PJ are employees of Thermo Fisher Scientific. All other authors declare no competing interests.

Data sharing

The UKHSA welcomes applications from organisations looking to use these data, and all applications will be rigorously reviewed using an objective, standards-based process. Potential applicants should contact DataAccess@ukhsa.gov.uk.

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