Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

An Overview of SARS-CoV-2 Molecular Diagnostics in Europe

Emma Davies, BSc, MSc, DClinSci, FRCPath[a,*],
Hamzah Z. Farooq, MBChB, MRCP, MSc, DipRCPath[a,b],
Benjamin Brown, BSc, MSc, PhD[a], Peter Tilston, BSc, MSc[a],
Ashley McEwan, BSc, MSc[a], Andrew Birtles, BSc, MSc, PhD[a],
Robert William O'Hara, BSc, PGCert, MSc, PhD, FIBMS[a],
Shazaad Ahmad, BMedSci, BMBS, MSc, FRCPath[a],
Nicholas Machin, BMedSci, BMBS, MSc, FRCPath[a],
Louise Hesketh, BSc, PhD, FRCPath[a], Malcolm Guiver, BSc, PhD, FRCPath[a]

KEYWORDS

• SARS-CoV-2 • COVID-19 • RT-PCR • Molecular diagnostics • CE Marking

KEY POINTS

- The COVID-19 pandemic has led not only to an influx of new molecular diagnostics but also a drive to modify existing technologies to allow the testing of thousands of patients daily over a variety of settings.
- The need for rapid turn-around times for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) testing for public health actions and patient care has led to the necessity for synchronously using multiple assays and platforms.
- Testing solutions exist for any scale of SARS-CoV-2 testing strategy.
- Overall SARS-CoV-2 molecular diagnostics seem to perform well; however, market saturation has left peer-reviewed real-world data lacking.
- With these new developments, diagnostic testing regulations for SARS-CoV-2 are paramount to aid manufacturers in achieving assay performance and for laboratories to use as a tool alongside local verification to determine the suitability of assays and platforms for use in future epidemics.

Funding: None declared.
Conflict of interest: None declared.
[a] Department of Virology, UK Health Security Agency, Manchester Foundation Trust, Oxford Road, Manchester M13 9WL, UK; [b] Department of Infectious Diseases and Tropical Medicine, North Manchester General Hospital, Manchester Foundation Trust, Manchester, UK
* Corresponding author. Department of Virology, UK Health Security Agency, Manchester Foundation Trust, Oxford Road, Manchester M13 9WL, UK.
E-mail address: emma.davies@mft.nhs.uk

Clin Lab Med 42 (2022) 161–191
https://doi.org/10.1016/j.cll.2022.02.005 **labmed.theclinics.com**

INTRODUCTION

An emerging viral pneumonia of unknown etiology was detected in patients from several health care facilities in the city of Wuhan in China on 30 December 2019.[1] A novel coronavirus was identified initially termed "2019-nCoV" and designated as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) with the clinical disease termed "coronavirus infectious disease-19" (COVID-19).[2–5] It has overwhelmed health care systems globally due to rapid asymptomatic spread and lethality leading the World Health Organization (WHO) to declare a COVID-19 pandemic on 11 March 2020.[6–8]

CLASSIFICATION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2, VIRION, AND GENOME

SARS-CoV-2 is a betacoronavirus and one of the seven known members of the Coronaviridae family.[4,9] It is an enveloped positive-strand RNA virus (single linear RNA segment) with a genome length of 29,881 bp (GenBank no. MN908947). Its genome has 14 open reading frames (ORFs), which encode for 28 different proteins—4 structural proteins such as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins; 16 nonstructural proteins (NSP 1–16); and 8 accessory proteins as shown in **Table 1**.[10]

The genome commences with a 5′ untranslated region (UTR), then the replication complex (ORF1a and ORF1b) followed by the four structural proteins and 3′ UTR, ending with nonstructural ORFs and a poly(A) tail.[10,11] ORF1a contains 10 NSPs, while ORF1b contains 16 NSPs. The combination of ORF1a and ORF1b codes for polyproteins pp1a and pp1b that form the viral replication complex.[10,11] Structurally, the RNA genome is bound by the N protein, while the S, E, and M proteins together create the double-layered lipid viral envelope. The principle genes of diagnostic significance are the RdRp (NSP-12), various ORF1ab regions, and the viral structural proteins (S, E, and N).[10]

HISTORY OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 MOLECULAR DIAGNOSTICS

The early sequencing of the SARS-CoV-2 genome and subsequent distribution of the genome sequence via Global Initiative on Sharing Avian Influenza Data (GISAID) enabled the development of nucleic acid amplification tests (NAATs), which became the cornerstone for the diagnosis of SARS-CoV-2. Although that is not the only molecular diagnostic technique, real-time polymerase chain reaction (RT-PCR) has become the mainstay across Europe with only limited use of other molecular techniques such as transcription-mediated amplification (TMA) or CRISPR.[12,13] One of the first published RT-PCR assays originated from Europe in January 2020 with primer probe sets targeting the E, N, and RdRp genes.[14] The RdRp assay included a Pan Sarbecco probe that detected SARS-CoV-1, SARS-CoV-2, and Bat-SARS-related-CoV with a second probe specific to SARS-CoV-2 leading to the recommendation of using the E gene assay as the first-line screening tool, followed by confirmatory testing with the RdRp gene assay.[14] A further assay was quickly developed by the Centers for Disease Control and Prevention (CDC) targeting multiple regions of the N gene, which has become the baseline assay for several commercially available molecular diagnostic tests.[15–18]

DIAGNOSTIC TESTING REGULATIONS IN EUROPE

At the start of the COVID pandemic, in vitro medical devices (IVD), including NAAT-based systems and assays, needed to comply with European Union Directive 98/79/EC In Vitro

Table 1
Table showing SARS-CoV-2 structural and nonstructural proteins and their respective functions

Gene	Protein	Function	References
Structural protein			
Spike (S)	S	Binds to Angiotensin-Converting Enzyme 2 (ACE2) receptor and heparan sulfate for viral entry	111
Envelope (E)	E	Virion structure	112
Membrane (M)	M	Virion structure	112
Nucleocapsid (N)	N	Contains genome; interferes with translation and cell cycle of the host cell.	113
Nonstructural protein (NSP)			
ORF1a *ORF1b*	NSP-1	RNA processing and replication	114
	NSP-2	Modulation of survival signaling pathway of host cell	115
	NSP-3	Possibly separates translated protein	116
	NSP-4	Contains transmembrane domain 2 (TM2) and modifies ER membranes	117
	NSP-5	Polyprotein replication	118
	NSP-6	Presumptive transmembrane domain	119
	NSP- 7 and NSP-8	Increases the combination of NSP-12 and template-primer RNA	120
	NSP-9	ssRNA-binding protein	120
	NSP-10	Cap methylation of viral mRNAs	121
	NSP-11	Unknown	122
	NSP-12	RNA-dependent RNA polymerase (RdRp)	123
	NSP-13	Binds with ATP and the zinc-binding domain - required for replication and transcription	124
	NSP-14	Proofreading exoribonuclease domain	125
	NSP-15	Mn(2+)-dependent endoribonuclease activity	126
	NSP-16	2′-O-ribose methyltransferase	127
	ORF 3a	Ion channel protein—affected cytokine response	128
	ORF 6	Inhibits antiviral interferon response	129
	ORF 7a	Inhibits antiviral interferon response and STAT1 phosphorylation	130
	ORF 7b	Inhibits antiviral interferon response, STAT1, and STAT2 phosphorylation	121
	ORF 8	Inhibits antiviral interferon response	131

This table also breaks down the components of *orf1ab* complex.
(*Adapted from* Suryawanshi and colleagues 122 (2021) and Wang and colleagues 132 (2020)).

Diagnostic Directive (IVDD) and bear a **Conformitè Europëenne (**CE) symbol as proof, to be marketed in European Union (EU) and European Free Trade Association countries and Turkey and the United Kingdom.[19,20] CE marking required the manufacturer to have verified compliance with legal requirements and prepared an EC declaration of conformity containing the device performance and safety data.[21] This allowed the device to be CE marked if it was intended for use by health care professionals although specific national requirements may also have been required.[19] Although the United Kingdom left the EU in 2020, it will still accept CE-marked kits until 2023 when the UK Conformity Assessed mark will be required to market IVDs in the United Kingdom.[22] Under Directive 98/79/EC, devices could also be granted emergency market access in the interest of health protection, such as in the COVID-19 pandemic; this required a derogation to be issued by the competent authority of a country allowing temporary marketing of a device without a full declaration of conformity, which was valid only for that nation.[19,21]

As of May 2021, Directive 98/79/EC was replaced in the EU by Regulation (EU) 2017/746, which expands the risk-based device classification system alongside a requirement for device assessment by independent third parties and confirmation of test performance by EU reference laboratories before a CE mark is awarded.[23] All products currently on the market that comply with the old legislation will have to recertify according to the new regulations.[23,24] Regulation (EU) 2017/746 still allows the national emergency market access of IVDs in the interest of protection of health if the derogation is issued by the country's competent authority.[23] This change in regulation brings CE marking more in line with the more stringent Food and Drug Administration (FDA) approval process, which requires devices to be tested by clinical trial and licensed only for use in specific circumstances.[25] On 17 June 2021, the UK government announced the intention to introduce a mandatory validation scheme initially for COVID-19 diagnostics to expand to cover all devices sold in the United Kingdom. This process would require manufacturers to provide a minimum set of standard performance data, which would undergo independent verification by specially commissioned laboratories. If successfully introduced, it would be a criminal offense to market devices that have failed or not undergone this mandatory validation in the United Kingdom under the Medicines and Medical Devices Act 2021.[26]

The above pieces of legislation along with the European Commission's guidelines for the Current Performance of COVID-19 Test Methods and Devices and Proposed Performance Criteria state the performance characteristics for IVDs, which includes but is not limited to analytical and diagnostic sensitivity and specificity, limits of detection (LODs), and expected values in normal and affected populations.[19,23,27] No required values for these characteristics are published in these documents although common specifications are planned.[24] A list of CE-marked COVID-19 IVDs is maintained at the European commission's Joint Research Centre In Vitro Diagnostic Devices and Test Methods Database.[28] As of 08/06/2021 325 CE-marked NAATs exist in this database originating from 240 unique manufacturers with 31 countries of origin. This database lacks key performance criteria for a significant number of entries including 120 tests with no stated LOD, 226 with no analytical sensitivity, 209 with no analytical specificity, and 200 with no clinical accuracy data. The entrance of many nontraditional manufacturers to the market has fueled a lack of peer-reviewed publications that make assessment of real-world performance difficult. An improved and standardized approach to market regulations would be welcomed as at present local validations/verifications of diagnostics are hugely important in ensuring the suitability of test selection for the intended purpose.

In addition to CE marking, the WHO and national bodies such as the UK Medicines and Healthcare products Regulatory Agency (MHRA) have published target product profiles (TPPs) that outline performance characteristics that a test must meet to be considered successful for its intended use.[29–32] WHO and MHRA TPPs outline "acceptable" and "desirable" characteristics including ranges for parameters such as analytical sensitivity/LOD and clinical sensitivity.[29–31] These documents are not legally binding but were developed to aid manufacturers in achieving assay performance that would be desired for use in the field. Equally these documents can be used by laboratories as a tool alongside local verification to determine the suitability of an assay for use. A selection of characteristics for NAAT-based tests is listed in **Tables 2** and **3** with the MHRA TPP showing much stricter acceptable criteria than the WHO criteria recommended for adoption by European Centre for Disease Prevention and Control (ECDC).[29–31,33]

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 MOLECULAR DIAGNOSTICS

The scale of testing required to manage the SARS-COV-2 pandemic has been unprecedented with extensive yet flexible testing strategies being key to protecting public health through prompt isolation of cases.[33,34] The United Kingdom has undertaken a dual-arm approach to testing with twice weekly at home rapid antigen tests being freely available and actively encouraged in the asymptomatic general population and in laboratory NAAT being used for more sensitive screening of all hospital admissions including day case and those with symptoms consistent with COVID-19.[35,36] The ECDC not only recommends the use of NAAT for all symptomatic cases but also acknowledges the role for rapid antigen tests in population screening.[33,34] The use of sensitive molecular diagnostic assays is important to the control of transmission. If SARS-CoV-2 infection is allowed to spread unchecked, the emergence of novel variants is likely to be enhanced as mutations in key genes continue to accumulate as part of the natural error-prone replication of RNA viruses. As mutations accumulate, it is not only possible that they can lead to increased pathogenicity or vaccine escape, but that they may also lead to detection failures in well-established diagnostic assays. It is now recommended that the presence of SARS-CoV-2 in clinical samples is determined through the detection of at least two distinct targets to mitigate this risk. The observation of the ThermoFisher S gene PCR assay failure in the United Kingdom for the B.1.1.7 Alpha variant, which would have led to significant numbers of false-negative tests being reported if this was being used as a single target assay, highlights the importance of a multi-target approach.[37]

To achieve testing on such an immense scale testing, a diverse approach has been required with laboratories often using multiple assays and platforms in unison. The following is by no means an extensive review of all diagnostic assays used in Europe but aims to provide an overview of some of the most common. Rapid antigen near patient point of care and isothermal amplification techniques are outside the scope of this review but will be covered elsewhere in this Clinics edition.

RAPID MOLECULAR DIAGNOSTICS

Rapid, commercial, cartridge-based sample-to-answer molecular diagnostic platforms for the detection of SARS-CoV-2 have fulfilled an important niche in point-of-care settings and clinical laboratories. They are simple to use, provide accurate results within 1–2 h, have minimal hands-on time, and permit on-demand testing of urgent specimens.

Table 2
Selected target product profile characteristics for point-of-care SAR-CoV-2 detection tests

	World Health Organization		Medicines and Healthcare Products Regulatory Agency	
Scope	**Desired**	**Acceptable**	**Desired**	**Acceptable**
Intended use	In areas with confirmed SAR-CoV-2 community-wide transmission. In suspected outbreak situations and to monitor trends in disease incidence.		Aid in the triage of current SARS-CoV-2 infection during active infection.	Aid in the triage of current SARS-CoV-2 infection during the acute phase of infection.
Target population	Patients with acute or subacute respiratory symptoms; suspicious symptoms and contact with confirmed or probable case/living in the area of cluster/community transmission.		People with/without SARS-CoV-2 clinical signs and symptoms if testing appropriate.	People with clinical signs and symptoms associated with SAR-CoV-2 infection.
Target user/ settings	Trained staff in health care facilities or community level or self-administrated.	Trained staff in health care facilities.	Trained health care professional (governed by professional standards authority). In primary/secondary/community health care settings and nonhealth care settings.	
Target analyte	SARS-CoV-2 only biomarker, for example, RNA, protein/antigen.	SARS-CoV-2 only biomarker. Assumption SARS-CoV-1 not circulating	Dual (or more) SARS-CoV-2 RNA or antigen targets.	Single (or more) SARS-CoV-2 RNA or antigen target.
Target type	Anterior nares, saliva/oral fluid, sputum	NP or OP or nasal swab, nasal wash, sputum	Sputum, saliva, or other method not using invasive swab	NP or OP, lower respiratory tract aspirate, BAL, nasopharyngeal wash/aspirate or nasal aspirate
Clinical sensitivity	≥90%	≥80%	>97% within confidence intervals of 93–100% [a]	>80% within 95% confidence intervals of 93–100% [a]
Clinical specificity	≥99%	≥97%	>99% within confidence intervals of 97–100% [b]	>95% within 95% confidence intervals of 90–100%b
Analytical sensitivity (LOD)	1×10^4 copies per ml or Ct≈>30	1×10^6 copies per ml or Ct ≈ 25–30	<100 SARS-CoV-2 copies/ml	<1000 SARS-CoV-2 copies/ml
Technical Failure rate	≤ 0.5%	< 2%	< 1%	< 5%
Turnaround time	≤ 20 min	≤ 40 min	< 30 min	< 2 h
Throughput	≥ 10/h per operator	≥ 5/h per operator	> 100 tests per unit per 12 h	> 6 tests per unit per 12 h

Abbreviations: BAL, bronchoalveolar; LOD, limit of detection; NP, nasopharyngeal swab; OP, oropharyngeal swab; Ct, Cycle threshold.
[a] Determined using at least 150 positive clinical samples covering a clinically meaningful range of viral loads.
[b] Determined using at least 250 negative clinical samples.

Table 3
Selected target product profile characteristics for high- and low-throughput diagnostic SAR-CoV-2 detection testing

	World Health Organization		Medicines and Healthcare Products Regulatory Agency	
Scope	**Desired**	**Acceptable**	**Desired**	**Acceptable**
Intended use	To detect the presence of virus components to diagnose or confirm acute and subacute SARS-CoV-2 infection.		Multiplex—determining current infection by detecting SARS-CoV-2 virus, differentiate other respiratory infections.	Determining current infection by detecting SARS-CoV-2 virus.
Target population	Patients with acute or subacute respiratory symptoms; suspicious symptoms and contact with confirmed or probable case/living in the area of cluster/community transmission.		People with/without clinical signs associated with SARS-CoV-2 infection.	People with clinical signs associated with SAR-CoV-2 infection.
Target settings/ users	High volume: reference laboratories/district hospitals/ mobile laboratories. Laboratory technicians. Low volume: outpatient clinics, point of care or near-patient settings. Laboratory technicians/health care workers.		Health care and medical laboratories. Trained health care professional (governed by professional standards authority) and suitably trained and assessed lab technician or scientist.	
Target analyte	Must have at least one target specific for SARS-CoV-2 RNA or protein/antigen.		Dual (or more) SARS-CoV-2 RNA. Multiplex panel for a range of infectious respiratory viruses.	Single SARS-CoV-2 RNA.
Target type	Samples amenable to self-collection: saliva/oral fluid, stool; inactivated samples.	NP or OP or nasal swab. Washes-nasal, oropharyngeal, BAL. Sputum	Oral fluid	NP or OP, lower respiratory tract aspirate, BAL, nasopharyngeal wash/aspirate, or nasal aspirate.
Clinical sensitivity	≥98%	≥95%	>99%. 95% two-sided confidence interval > 97%[a]	>95%. 95% two-sided confidence interval > 90%[a]
Clinical specificity	≥99%	≥99%	>99%. 95% two-sided confidence interval > 97%[b]	>95%. 95% two-sided confidence interval > 90%[b]
Analytical sensitivity (LOD)	1×10^2 copies per ml in upper/lower respiratory tract specimens, stool	1×10^3 copies per ml in any respiratory tract specimen.	≤100 SARS-CoV-2 copies/ml	≤1000 SARS-CoV-2 copies/ml

(*continued on next page*)

Table 3
(*continued*)

	World Health Organization		Medicines and Healthcare Products Regulatory Agency	
Scope	**Desired**	**Acceptable**	**Desired**	**Acceptable**
Technical failure rate	NA	NA	<0.2%	<1%
Turnaround time	< 45 min	< 4 h	< 90 min	< 5 h
Throughput	High volume: 200–500 tests in 4 h. Low Volume: 6 patients in 45 min	High volume: 50–150 tests in 4 h. Low volume: 1–4 patients per 45 min	> 200 tests in unit per 4 h	> 50 tests in unit per 4 h

Abbreviations: BAL, bronchoalveolar; LOD, limit of detection; NP, nasopharyngeal swab; OP, oropharyngeal swab.
[a] Determined using at least 150 positive clinical samples covering a clinically meaningful range of viral loads.
[b] Determined using at least 250 negative clinical samples.

Table 4
An overview of rapid, cartridge-based, sample to answer SARS-CoV-2 molecular tests

Test Name	Manufacturer	Target 1	Target 2	Internal Control	Platform	Maximum Sample Capacity	Platform Run Time (min)	Sample Input Volume (uL)
Xpert Xpress SARS-CoV-2 Xpert Xpress SARS-CoV-2/Flu/RSV	Cepheid	N2	E	Manufacturer SPC	GeneXpert Dx and GeneXpert Infinity	2–16 (Dx) or Up to 80 (Infinity)	45	300
BioFire® Respiratory Panel 2.1 plus (RP2.1 plus)	BioMerieux	S	M	*Schizosaccharomyces pombe*	FilmArray 2.0 and FilmArray Torch	2–12	45	300
Cobas Liat SARS-CoV-2 and Influenza A/B	Roche	ORF1 a/b	N	Manufacturer SPC	Cobas Liat	1	20	200
Novodiag COVID-19	MobiDiag	ORF1 a/b	N	RNAse P and Manufacturer SPC	Novodiag	4–16	60	500
VitaPCR SARS-CoV-2 VitaPCR SARS-CoV-2/Flu AB	Credo Diagnostics Biomedical Pte	N	N	β-globin	VitaPCR	1	20	30[a]
Aries SARS-CoV-2	Luminex	ORF1a/b	N	RNAse P	Aries	12	120	200
GenomEra SARS-CoV-2 GenomEra SARS-CoV-2, Flu A/B+ RSV	Abacus Diagnostica	RdRP	E[b]	MS2	GenomEra CDX	4	70	35[c]
QIAstat-Dx Respiratory SARS-CoV-2 Panel	Qiagen	ORF1 a/b (RdRp)	E	MS2	QIAstat Dx Analyzer	1	70	300
GenMark ePlex SARS-CoV-2 GenMark ePLex Respiratory Pathogen Panel 2 (RP2)	GenMark Dx	N	N	Manufacturer SPC	ePlex	3 (ePlex NP) to 24 (ePlex 4 Tower)	90	200

Abbreviations: N, nucleocapsid; E, envelope protein; S, spike glycoprotein; M, membrane protein; ORF1 a/b, open reading frame 1 a/b; RdRP, RNA-dependent RNA polymerase; SPC, sample process control.

[a] 30 uL lysate (lysis buffer containing sample).

[b] GenomEra SARS-CoV-2 contains E gene. GenomEra SARS-CoV-2, Flu A/B+ RSV contains only RdRP.

[c] 50 uL of sample is heated and mixed with 1 mL of lysis buffer, after which 35 uL of processed sample is loaded onto the test chip.

An overview of the main sample-to-answer platforms is presented in **Table 4**. These single-use tests often automate nucleic acid extraction, purification, amplification, detection, and interpretation of results. All the platforms presented are internally controlled yet only three use an endogenous sample control, which monitors for an adequately taken sample and sample degradation. Independent studies evaluating the performance of rapid RT-PCR tests have varied with few head-to-head comparisons although evaluations of these platforms are more extensively published due to their widespread use in non-specialist laboratories.

Unlike other applications, the rapid testing platforms exhibit significant variation in the technologies used. Cepheid Xpert Xpress, QiaStatDx, and VitaPCR SARS-CoV-2 rely on classic multiplex RT-PCR. Novodiag COVID-19[38] is unique in its use of qPCR and microarray technology for the detection of SARS-CoV-2. GenomEra SARS-CoV-2[39] and GenomEra SARS-CoV-2 with Flu A/B+ RSV[40] use multiplex RT-PCR performed on chips. BioFire Respiratory Panel 2.1 plus (RP2.1plus)[41] achieves extensive multiplexing through an initial RT-PCR step before target amplification using numerous monoplex PCR reactions, which are detected using endpoint melt curve analysis. GenMark ePlex SARS-CoV-2[42] and GenMark ePlex Respiratory Pathogen Panel 2 (RP2)[43] use RT-PCR in combination with electrowetting and GenMark's eSensor technology involving electrochemical detection rather than optical detection of fluorescence.

Aside from the variation in technologies, the rapid testing platforms also offer detection of the widest range of pathogens. With the exception of Luminex Aries, SARS-CoV-2 can be detected in isolation or in combination with influenza as a minimum.[44,45] BioFire RP2.1plus[41] detects 23 respiratory pathogens, GenMark ePlex RP2[43] detects 25 respiratory pathogens, and the QIAstat-Dx Respiratory SARS-CoV-2 Panel[46] detects 22 respiratory pathogens.

Xpert Xpress SARS-CoV-2[47] is the most widely evaluated rapid test with a recent systematic review and meta-analysis encompassing 1734 subjects determining a pooled sensitivity of 99% (97–99, 95% CI) and a specificity of 97% (95–98, 95% CI).[48] Reported sensitivities for other platforms range from 90 to 100% with particular issues noted for samples with high cycle threshold (Ct) values in some studies.[45,49–52] Fitoussi and colleagues (2021)[49] found a VitaPCR SARS-CoV-2 sensitivity of 60% for samples that were positive at Ct > 33 using a comparator N gene assay; however, VitaPCR involves no formal RNA extraction and purification that may account for this poor performance.[49] All tests in **Table 4** were shown to be near 100% specific except for the VitaPCR SARS-CoV-2 and QIAstat-Dx.[45,52] The VitaPCR gave a specificity of 94.7% in one study due to its increased sensitivity over the comparator assay, and a second study showed an improved sensitivity of 99%.[45,49] The QIAStat-Dx gave a specificity of 93% compared with a WHO-recommended RT-PCR.[52]

Evaluations often used small sample sets, due to a limited availability of reagents and used various SARS-CoV-2 reference controls, making LOD comparisons difficult. Reported LODs varied from 100 copies/ml for Xpert Xpress SARS-CoV-2 to 3000 genome copy equivalents for the Aries SARS-CoV-2.[53] Several platforms fail to achieve the MHRA TPP "acceptable" LOD criteria of 1000 copies/ml; GenomEra SARS-CoV-2, Flu A/B+ RSV at 2857 copies/mL,[40] Novodiag COVID-19[38] at 1815 copies/mL when using collection devices other than the provided medium nucleic acid amplification test;[54] and both the GenMark ePlex SARS-CoV-2[42] and the QIAstat-Dx Respiratory SARS-CoV-2 Panel[46] at 1000 copies/ml.

The main limitations of the rapid sample-to-answer platforms include their high cost per test and low sample throughput. Moreover, despite their low complexity, rapid platforms are not infallible, and they are sensitive molecular tests that can be

compromised without meticulous sample processing and good laboratory practice. Notably, BioFire and ePlex platforms do not output Ct values, meaning there is no indication of SARS-CoV-2 viral burden that can be of interest to the clinician as higher viral loads have been associated with increased SARS-CoV-2 mortality.[55]

STAND-ALONE REAL-TIME POLYMERASE CHAIN REACTION KITS

One of the biggest barriers to the implementation of SARS-CoV-2 testing in non-specialist laboratories early in the pandemic was the availability of the correct equipment to enable the rapid introduction of testing. The solution to this problem for many manufacturers was the rapid introduction to the market of stand-alone assays encompassing kits, which include the reagents necessary for reverse-transcription PCR, including controls, but that are not tied to a specific extraction or PCR platform. They offer flexibility over more "closed" systems as they can potentially be run on existing instrumentation, precluding the requirement for purchasing new and often expensive equipment. Use of such reagents requires more extensive validation than end-to-end systems, and the onus on providing this validation, including sample preparation and the compatibility of any instrumentation with a particular kit, will fall on the individual laboratory. Some suppliers provide details of compatible platforms, but many do not, and it is this lack of data that have allowed many substandard kits to enter the market. Over 200 CE-marked manual RT-PCR kits are listed on the COVID-19 In Vitro Diagnostic Medical Devices database,[28] a selection of which are shown in **Table 5** along with some of their main attributes.[18,56–73]

Kit formats are broadly similar and include minimal necessary reagents (primer/probe mixes, controls). Reagents may be provided either lyophilized or "wet" most commonly in tubes but also as eight-well strips. Although earlier kits relied on a single viral gene target, these have now been largely superseded by dual or triple target assays that focus on some combination of the E, N, S, and Orf1a genes. Although this has made the assays more robust in dealing with the emergence of novel SARS-CoV-2 variants, it has also complicated the interpretation of results when some gene targets fail to amplify. Furthermore, most kits supply an internal control (IC), which may be either endogenous (eg RNase P)[18,58,64,65,71] or exogenous (eg MS2),[62,63,68] which can be used either as full process controls or solely as PCR controls. Some kits include both endogenous and exogenous ICs[72] although some fail to disclose the IC origin.[52,56,57,59,60,67–70,73]

The number of tests per kit ranges from 48 to 4800 allowing for a wide range of throughputs although this will also depend on the number of wells required per sample and whether they are being tested in 96- or 384-well format. Many assays exploiting RT-PCR can typically use up to four different fluorescent reporter dyes, including the IC, but others are not so comprehensively multiplexed and require two or even three wells for each sample. At least one kit (Menarini)[74] uses melt curve analysis in preference to hydrolysis probes, negating the requirement for multiple fluorescent reporter dyes. Although not shown in **Table 5**, many SARS-CoV-2 kits are also formulated as multiplexes with other respiratory viruses, most commonly influenza and respiratory syncytial virus (RSV), for example, Altona,[75] Viasure,[76] and ThermoFisher.[77] This will usually require the addition of an extra well for each sample and/or the use of a single dye for multiple gene targets of the same virus. The actual throughput for these assays will depend heavily on the extraction and PCR equipment chosen for use and the level of automation. Use of an automated end-to-end system like the Roche FLOW could produce in excess of 1000 results in a 24hr period from experience in our local laboratory.

Table 5
An overview of stand-alone RT-PCR suppliers and kits available in the EU. Details are taken from company websites and/or accompanying literature

Supplier	Kit Name	Target 1	Target 2	Target 3	Internal Control	No' of Tests/ Kit	Compatible Platforms	Analytical Sensitivity	References
Altona	RealStar® SARS-CoV-2 Virus RT-PCR Kit 1.0	E	S		Manufacturer SPC	384/4800	Bio-Rad CFX96, Bio-Rad CFX96 deep-well, ABI QuantStudio, ABI 7500, Roche LightCyler 480, Qiagen Rotor-Gene Q	E = 0.025 pfu/mL S = 0.014 pfu/mL	56 66 67 68 69 70
Anatolia Geneworks /Launch	Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v4	Orf1ab	N	E	RNAse P	50/100	Not stated	orf1ab = 0.86 copies/ul N = 0.82 copies/ul E = 1.02 copies/ul	71
Biomaxima	SARS-CoV-2 Real-Time PCR LAB-KITTM	Orf1ab	N		Manufacturer SPC	96 (12 × 8 well strips)	"Open PCR systems"	10 copies/reaction	No literature found[a]
BioMerieux	Argene SARS Cov-2 R-Gene	N	RdRp	E	Endogenous (HPRT1) and Manufacturer SPC	120	ABI 7500, ABI QuantStudio5, Roche LightCycler 480, Bio-Rad CFX96, Qiagen Rotor-Gene Q	0.43 TCID50/mL (equivalent to 380 copies/mL).	72
Bio-Rad	Reliance SARS-CoV-2 RT-PCR Assay Kit	N1	N2		RNAse P	200	Bio-Rad CFX96, ABI 7500	125–250 copies/ml	No literature found[a]
Clonit	Quanty COVID-19 v2 (quantitative)	N1	N2		RNAse P	96	ABI 7500, Qiagen Rotor Gene Q, Bio-Rad CFX96	Not stated	No literature found[a]
Clonit	COVID 19 HT Screen (qualitative)	N1	N2		Manufacturer SPC	96	ABI 7500, Qiagen Rotor Gene Q, Bio-Rad CFX96	Not stated	No literature found[a]

Euroimmun	EuroRealTime SARS-CoV-2	Orf1ab	N		Manufacturer SPC	25–1000	Roche LightCycler 480, ABI 7500, Bio-Rad CFX 96, Qiagen Rotor-Gene Q, qTower 3	1 copy/ul	73
Genetic Signatures	EasyScreen SARS-CoV-2 Detection Kit	N	E		Manufacturer SPC	96	ABI Quantstudio 5	Not stated	57
IDT	2019-nCov CDC Assay	N1	N2		RNAse P	96	ABI 7500	1–3 copies/ul	18 58
Menarini	Corona MELT	Orf1ab	Orf1ab		Human GADPH	100	Most commercial Real Time PCR instruments	20 copies/ reaction	No literature found[a]
Perkin Elmer	SARS-CoV-2 Real-time RT-PCR Assay	Orf1ab	N		MS2	48	Bio-Rad CFX96/385, ABI 7500, ABI QuantStudio, qTower 3	20 copies/ml	No literature found[a]
Primerdesign	genesig® COVID-19 2G Real-Time PCR assay	Orf1ab	S		Manufacturer SPC	96	ABI 7500, Bio-Rad CFX Connect, Roche LightCycler 480, genesig® q32	0.4 copies/ul	69 59 60
RIDA®GENE	SARS-CoV-2	E			Manufacturer SPC	100/200	RIDA CYCLER, Roche LightCycler 480, Mx3005P, ABI 7500, Bio-Rad CFX96, Qiagen Rotor-Gene Q	50 copies/ reaction	61
Seegene	Allplex 2019-nCOV	RdRp	N	E	Manufacturer SPC	50/100	Roche LightCycler 480 (minimum)	1–4 copies/ul	67 69 70 62
Serosep	Respibio SARS-CoV-2	Not stated			Not stated	96	Roche LightCycler 480, ABI 7500	Not stated	No literature found[a]

(*continued on next page*)

Table 5
(*continued*)

Supplier	Kit Name	Target 1	Target 2	Target 3	Internal Control	No' of Tests/ Kit	Compatible Platforms	Analytical Sensitivity	References
Thermofisher	TaqPath COVID-19 CE-IVD RT-PCR Kit,	S	N	orf1ab	MS2	Up to 1000 (96- and 384-well format)	ABI 7500, ABI Quantstudio 5	10 genome copy equivalents/ reaction	68 62 63
TIBMOL BIOL	Dual Target SARS	N	E		UBC Human mRNA	96	Roche LightCycler 480	Not stated	64 65
ViaSure (CerTest Biotech)	SARS-CoV-2 Real Time PCR	Orf1ab	N		Not stated	96	"Most open PCR systems"	1–10 copies/ reaction	18
VirCell	SARS-CoV-2 Real Time PCR Kit	N	E		RNAse P	48	"Most open PCR systems"	3–5 copies/ reaction	No literature found[a]

Abbreviations: N, nucleocapsid; E, envelope protein; S, spike glycoprotein; ORF1 a/b, open reading frame 1 a/b; RdRP, RNA-dependent RNA polymerase; SPC, sample process control.

[a] Indicates that using the kit name in combination with either "COVID-19″ or "SARS CoV-2″ as the search term in PubMed and Google Scholar yielded no significant results.

Owing to the pressure to manufacture diagnostic kits rapidly as the pandemic took hold, much of the technical and clinical validation data used minimal data sets. Unlike the rapid platforms that are in widespread use, peer-reviewed literature is sparse for many stand-alone kits and in some cases completely absent. For those referenced assays in **Table 5**, the LOD was most commonly in the range of 1–20 copies/reaction although this was liable to small variations depending on the extraction and eluate volume and the volume of eluate used in the PCR. When comparisons between kits using clinical samples or External Quality Assurance (EQA) samples were performed, most kits performed comparably with only small variations in results between the Altona,[52,56,66–70] Integrated DNA Technologies (IDT),[18,58] Seegene,[62,67,69,70] TaqPath,[62,63,68] Viasure,[18] and Tib MolBiol kits.[64,65] Specificity was 100% in virtually all cases.

Stand-alone kits offer a convenient alternative to more closed systems allowing rapid implementation on existing equipment. However, despite a broad agreement in the performance of these assays on clinical specimens, the sheer number of kits available means that in-house validation is essential before implementation as a clinical service.

LOW-THROUGHPUT TESTING PLATFORMS

The use of stand-alone PCR kits is not always an attractive option for laboratories, particularly if the existing molecular diagnostic infrastructure is not in place. Manufacturers identified a niche in the market for automated low-to-medium input end-to-end solutions, which could be easily introduced to laboratories with minimal molecular diagnostic experience. All platforms assessed here use multiplex RT-PCR with all assays containing an IC except the Virokey SARS-CoV-2, which contains neither an endogenous nor manufacturer-provided IC (**Table 6**).[78] False-negative results will not be identified by the failure to include an IC to demonstrate either sample adequacy or PCR failure. The Qiagen NeuMoDx has the best throughput of these systems at 435 samples in 24hr and also has the advantage of being a true random access platform with a quick time to result of only 1hr 25 min.[79]

Peer-reviewed literature for these platforms is significantly lacking over all other investigated areas with most performance data presented here being sourced from the manufacturer's literature. The BD MAX system can use a variety of kits from different manufacturers including SARS-CoV-2 in isolation or with other respiratory pathogens such as influenza. The BD MAX SARS-CoV-2 assays, including the ViaSure SARS-CoV-2 N1 + N2 assay, have repeatedly shown 100% sensitivity but the specificity of greater than 95% both in manufacturers post-market surveillance and in real-world data. Fears around the production of false-positive results led the FDA to release a product notice recommending confirmation of all positive results generated by the BD MAX; however, both of the assessed assays are based on the CDC N gene assay, which has been shown to be highly sensitive.[16,17,80] The Amplidiag COVID-19 assay was highly sensitive showing greater than 98% agreement compared directly with Cobas 6800 SARS-CoV-2. All other assessed platforms as shown in **Table 6** were also found to have acceptable sensitivity and specificity of greater than 96% based on manufacturer's data only.[78,79,81–84]

All assessed platforms were shown to have good analytical sensitivity as outlined in **Table 6** with the exception of Aus Diagnostics SARS-CoV-2, influenza, and RSV, which has an LOD on 2150 to 4325 copies/ml.[82] Real-world testing of the Amplidiag COVID-19 also highlighted a failure to detect an EQA sample at 3300 copies/ml suggesting the manufacturer published LOD of 313 copies/ml may not be reliable.[83] Local

Table 6
An overview of low- to mid-throughput end-to-end testing platforms for SARS-CoV-2

Supplier/ Platform	Assay	Target 1	Target 2	Internal Control	Analytical Sensitivity	Batch Size	Platform Run Time	Throughput 24hr	References
Mobidiag Amplidiag Easy	Amplidiag COVID-19	*Orf1*	N	RNAse P	313 copies/ml	48	3.5 h	288	133 134 135
BD MAX	BD SARS-CoV-2	N1	N2	RNAse P	640 genomic copy equivalents	24	2.5 h	216	17
EliTech Elite InGenius	SARS-CoV-2 PLUS ELITe MGB Kit	Orf1ab	Orf8	RNAse P	111 genomic copy equivalents	12	2.5 h	108	81
ViaSure (CerTest Biotech)	SARS-CoV-2 (N1 + N2) – BD MAX	N1	N2	RNAse P	$\geq$ 5 genome copies per reaction	24	2.5 h	216	16
Vela Diagnostics Sentosa	ViroKey SARS-CoV-2 RT-PCR Test v2.0	Orf1a	N	None	200 genome equivalents/ml	46	4 h	276	78
Aus Diagnostics HighPlex 24	SARS-CoV-2 influenza and RSV 8-well	Orf1	Orf8	Endogenous and Manufacturer SPC	2150–4325 copies/ml	24	4.5 h	120	82 136
NeuMoDx™	NeuMoDx™ SARS-CoV-2 Assay	Nsp2	N	Manufacturer SPC	200[11]	Random Access	1 h 25 min	435	84

Abbreviations: N, nucleocapsid; ORF1 a/b, open reading frame 1 a/b; Orf 8, open reading frame 8; SPC, sample process control.

verification of the manufacturer's claims is important before the introduction of any test into routine use to ensure discrepancies such as this are detected.

The expected 24hr throughput for these systems is modest, and these systems are likely to be sited in laboratories that do not undertake 24/7 working meaning their full potential cannot be met. Although this may be the case, these automated solutions can offer easy-to-use solutions for laboratories with limited molecular experience. This has been important in providing the ability to decrease time to result over sending samples to specialist reference laboratories for testing, which in turn can reduce transmission risk particularly in health care settings.

HIGH-THROUGHPUT TESTING PLATFORMS

Several high-throughput platforms have been introduced for the detection of SARS-CoV-2 RNA offering end-to-end automated testing of samples from nucleic acid extraction through to amplification and detection. The introduction of high-throughput screening platforms into laboratories can improve laboratory efficiency and turnaround times while reducing staff hands-on time[85] and facilitating a substantial increase in a testing capacity. The main high-throughput testing platforms and associated assays are listed in **Table 7**. All are RT-PCR-based assays except the Hologic Aptima SARS-CoV-2 assay that use TMA. All assays listed use a minimum of two different SARS-CoV-2 targets to reduce the risk of false negatives due to primer/probe mismatches caused by sequence variability.[86] Multiple comparisons between the high-throughput platforms and standard RT-PCR demonstrate a high level of diagnostic performance. The Panther Fusion had an overall agreement of 96.4% compared with the Roche Cobas 6800 SARS-CoV-2 assay[87] with a similar finding in a separate study.[88] An agreement of 98.3% was found when comparing the Cobas to the Abbott Alinity M SARS-CoV-2 AMP,[89] and in a three-way comparison between these platforms and the Panther Fusion, the overall agreement was 99.7%.[90] When the TMA-based Aptima assay was compared with the Panther Fusion and rapid low-throughput BioFire Defense COVID-19 test, it produced a positive percent agreement of 98.7% compared with the consensus and a 100% agreement for negative results.[91]

Comparing analytical sensitivity is difficult due to differences in methods between studies, but generally all have high analytical sensitivities with LODs of 200 copies/ml or below, as collated from several studies and listed in **Table 1**. The TMA-based Aptima assay was shown to have a lower LOD when compared with standard RT-PCR,[13] although when compared directly against the Roche Cobas and Abbott m2000, the Cobas test had the lowest LOD,[84] a similar finding when the Cobas was directly compared with the Abbott m2000 and Panther Fusion.[92]

All systems offer a throughput of 1000 samples or more in a 24hr period. The highest throughput systems are the Roche Cobas 8800 system and the recently introduced Thermofisher Amplitude running the Taqpath COVID-19 assay, which claims a very high throughput of 8000 samples from a single platform over 24 hours. The Taqpath COVID-19 assay has been evaluated as a standard RT-PCR[62] assay, but no published data exist for the diagnostic performance of the complete Amplitude system. Assays for these high-throughput platforms are being updated to include additional respiratory targets to meet the predicted increases in RSV and seasonal influenza infections once nonpharmaceutical interventions for COVID are removed.[93] These include the Roche Cobas SARS-CoV-2 and Influenza A/B for the 6800/800 systems, the Aptima SARS-CoV-2/Flu Assay for the Hologic Panther system, and the m RESP-4-PLEX ASSAY for the Abbott Alinity system.[94] The Cepheid GeneXpert infinity platform can

Table 7
An overview of high-throughput molecular diagnostic platforms for SARS-CoV-2

Platform	Assay	Target 1	Target 2	Target 3	Internal Control	Analytical Sensitivity SARS-CoV-2 RNA c/ml	Platform Run Time	Throughput 24hr	Loading	References
Abbott m2000	Abbott RealTime SARS-CoV-2	RdRp	N		Manufacturer SPC	53[92]	4 h	470	Batch	92
Abbott Alinity M	SARS-CoV-2 AMP Kit	RdRp	N		Manufacturer SPC (DNA)	50[90]	2 h 35 min to first results	1080	Random Access	90
Hologic Panther®	Aptima® SARS-CoV-2 Assay	Orf1Ab Region 1	Orf1ab Region 2		Manufacturer SPC	83–194[137,138]	3.5 h to the first result	1150	Batch	137 138
Hologic Panther Fusion®	Panther Fusion® SARS-CoV-2 Assay	Orf1Ab Region 1	Orf1ab Region 2		Manufacturer SPC	74–100[92,139,140]	2.4 h to first results	1440	Random Access	92 139 140
Roche Cobas® 6800	cobas® SARS-CoV-2	Orf1ab	E		Manufacturer SPC	<10–85[92,141]	3.4 h to first results	1440	Batch	92 141
Roche Cobas® 8800								4128	Batch	
Cepheid Infinity	Xpert Xpress SARS-CoV-2	N	E		Manufacturer SPC	100	50 min per cartridge	Up to 1920	Random Access	95
Thermofisher Amplitude	TaqPath COVID-19 HT	S gene	N	Orf1ab	MS2	N/A	3 h 30 min to first result	8000	Batch	No literature found

give users the option to run up to 80 Xpert Xpress SARS-CoV-2 cartridges simultaneously with no increase in run time over the smaller cepheid instruments making this a high-throughput low complexity solution for laboratory settings.[95]

SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 GENOTYPING

All viruses mutate, particularly RNA viruses, and the infection rate of SARS-Cov-2 on a large susceptible population has greatly increased the opportunity for mutations to occur. These mutations have led to variants of concern (VOCs) emerging with the potential of enhanced fitness, specifically toward increased transmissibility[96,97] and vaccine evasion.[98–102]

The first VOC (B.1.1.7—Alpha) was detected in the south of England and sequenced in September 2020.[103] Soon after, new VOCs were identified from various locations across the world, each VOC becoming a prominent strain within their area of origin.[104] Genomic sequencing is an invaluable tool in managing the pandemic due to its ability to detect unknown variations, which may indicate the emergence of a new VOC and the need for the development of new diagnostic assays. The United Kingdom currently sequences all SARS-CoV-2-positive samples where it is technically achievable; however, it can be slow, technically demanding, and currently has limited global availability.[34] One solution to identifying known SARS-CoV-2 lineages without the need for genomic sequencing is the development of real-time genotyping PCR assays.

Rapid real-time genotyping PCR assays usually target a single nucleotide polymorphism (SNP), with the most discriminatory targets often located within the S-gene. These types of mutations invariably lead to nonsynonymous amino acid substitutions. SNPs within this region can cause changes in the receptor-binding motif with successful variants retaining an increased affinity of the S-protein to the human angiotensin 2 receptor (ACE2).[103,105–107] Identification of these distinct mutations can be used as markers to detect specific VOC lineages.

It is often the case that one distinct mutation may be present in several VOCs. For example, the presence of the N501Y mutation alone can be distinctive of the B.1.1.7 lineage, but the N501Y is also present in the B.1.351 and P1 VOC alongside the E484 K and K417 N or K417 T mutations, respectively; although the E484 K mutation is also occasionally seen in the B.1.1.7 lineage. It is often necessary to assay multiple targets to reliably determine the likely SARS-CoV-2 lineage. The range of SNP assays used will need to be modified as the new VOC are identified through whole-genome sequencing strategies.

Public Health England currently uses the Applied Biosystems (Waltham, Massachusetts, USA) RT-PCR genotyping assay for the rapid detection of variants. This genotyping assay has a sufficient repertoire of target mutations to reliably cover all the major VOC currently recognized by the WHO and most of the variants of interest.[108,109] The current selection consists of 32 assays that can detect 30 SNPs and 2 deletions. Each assay is duplex in format detecting the mutant and the original SARS-CoV-2 reference/wild-type sequence on two different fluorescent dye layers. The high specificity of each assay target results in a significant reduction in the sensitivity, and it is advised by the manufacturer to only use extracted RNA from specimens with a CT of $\leq$30 where this information is available.[109] There are several VOC assays in development or in early stages of marketing as shown in **Table 8**, many of which exist in stand-alone format to allow a reactive and rapid introduction of new SNP assays to the market as dictated by circulating variants. Agena Bioscience has developed the MassARRAY SARS-CoV-2 Variant Panel capable of

Table 8
A small selection of SNP PCR assays available in Europe for the detection of SARS-CoV-2 variants of concern

Manufacturer	Assay	Targets	Variant	References
EliTech	SARS-CoV-2 Variants ELITe MGB® Kit	• S gene, E484 K • S gene, N501Y	Alpha	142
ViaSure (CerTest Biotech)	SARS-CoV-2 & UK Variant	• HV 69/70 s gene deletion	Alpha	143
Anatolia Geneworks/ Launch	Bosphore SARS-CoV-2 UK. Variant Detection Kit	• A570D • P681H • Y144del	Alpha	144
Thermofisher	TaqMan Custom SNP Assays	Bottom of Form • D215 G • D614 G • HV 69/70 s gene deletion • Y144del • E484 K • E484Q • F888 L • K417 N • K417 T • L18 F • L452 R • N439 K • N501Y • P681H • P681 R • S13I • S477 N • T20 N • V1176 F	Alpha Beta Gamma Delta Plus numerous variants of interest depending on combination used	109 108
TIBMOL BIOL	VirSNiP Assays	• H66D • A67 V • HV 69/70 s gene deletion • D253 G • K417 N • K417 T • L452 R • Y453 F • T478 K • E484 K • E484Q • N501Y • A570D • P681H • P681 R • F888 L • Q949 R • V1176 F	Alpha Beta Gamma Delta Plus numerous variants of interest depending on combination used	145

(*continued on next page*)

Table 8
(*continued*)

Manufacturer	Assay	Targets	Variant	References
Agena Bioscience	MassARRAY SARS-CoV-2 Variant Panel	• L452 R • E484Q • P681 R • T478 K • T19 R • P681H • N501Y • A570D • HV 69/70 s gene deletion • S982 A • T716I • Y144del • D80 A • D215 G • K417 N • E484 K • A701 V • L18 F • L242_L244del • Q677H • D253 G • L5F • T95I • S477 N • D80 G • S13I • W152 C • N439 K • K1191 N • Q493 K • I692 V • Y453 F • N501 T • Q677P	15 variants of interest including: Alpha Beta Gamma Delta	110

detecting 15 variants over 36 gene targets in a two-well multiplex end-point RT-PCR assay.[110]

The use of SNP genotyping assays for the detection of SARS-CoV-2 VOC can be an effective early warning system for emerging VOC within a population, with quicker turnaround times compared with genomic sequencing. Data produced from this method can help scientists to quickly predict the prevalence of a VOC within a given population and may provide evidence toward vaccine effectiveness for new variants when collated with data regarding new infections or hospitalizations.

SUMMARY

The COVID-19 pandemic will have a long-reaching impact on molecular diagnostic testing. The speed at which molecular diagnostics entered the market has been unrivaled with strategies suitable for all desired testing throughputs available within a few short months. The overall analytical and clinical accuracy data for solutions

marketed within Europe have generally been found to be satisfactory although published LODs can be variable. At the outset of the pandemic manufacturers, claims were not required to be independently verified in Europe, and outside the most used rapid or high-throughput testing platforms, peer-reviewed real-world data are sparse. Welcome changes to regulations for devices in Europe are on the horizon, but local laboratory validations will still play a key role in the future. With the increasing prevalence of new SARS-CoV-2 VOC and the need for enhanced surveillance, there is still potential for new developments in SARS-CoV-2 molecular diagnostics.

CLINICS CARE POINTS

- evere acute respiratory syndrome coronavirus-2 (SARS-CoV-2) required the rapid expansion of virological diagnostic techniques to ensure adequate testing capacity in the pandemic settings.
- Rapid, molecular diagnostic platforms fulfill an important niche in point-of-care settings and clinical laboratories. They provide quick accurate results require minimal hands-on time and permit on-demand testing of urgent specimens, which is pertinent for non-COVID patient care.
- High-throughput platforms improve laboratory efficiency and turnaround times while reducing staff hands-on time. This leads to an increase in the testing capacity of diagnostic laboratories to help meet the clinical demand throughout pandemics.
- The use of SNP genotyping assays for the detection of SARS-CoV-2 VOCs can be an effective early warning system for emerging VOCs within a population, with faster turnaround times compared with genomic sequencing. This can assist with public health surveillance and provide high-quality evidence toward vaccine effectiveness.

REFERENCES

1. ProMED-mail. Published Date : 2020-01-05 18 : 15 : 37 Subject : PRO/AH/EDR > Undiagnosed Pneumonia - China (HU) (03): Updates , SARS , MERS Ruled out , WHO , RFI Archive Number : 20200105 . 6872267.; 2020.
2. Zhang Y-Z. Initial genome release of novel coronavirus. Virological. Available at: https://virological.org/t/novel-2019-coronavirus-genome/319. 2020. Accessed June 23, 2021.
3. World Health Organization. Novel coronavirus (2019-NCoV) situation reports. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf?sfvrsn=20a99c10_4. 2020. Accessed June 23, 2021.
4. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5(4):536–44.
5. World Health Organization. Naming the coronavirus disease (COVID-19) and the virus that causes it. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it. 2020. Accessed June 23, 2021.
6. Tangcharoensathien V, Bassett MT, Meng Q, et al. Are overwhelmed health systems an inevitable consequence of covid-19? Experiences from China, Thailand, and New York State. BMJ 2021;372:n83.

7. Narain JP, Dawa N, Bhatia R. Health system response to COVID-19 and future pandemics. J Health Manag 2020;22(2):138–45.
8. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. Acta Biomed 2020;91(1):157–60.
9. Huang Y, Yang C, Xu X, et al. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 2020;41(9):1141–9.
10. Wu A, Peng Y, Huang B, et al. Genome composition and divergence of the novel Coronavirus (2019-nCoV) originating in China. Cell Host Microbe 2020;27(3):325–8.
11. Kim D, Lee J-Y, Yang J-S, et al. The architecture of SARS-CoV-2 transcriptome. Cell 2020;181(4):914–21.e10.
12. Datta M, Singh DD, Naqvi AR. Molecular diagnostic tools for the detection of SARS-CoV-2. Int Rev Immunol 2021;40(1–2):143–56.
13. Gorzalski AJ, Tian H, Laverdure C, et al. High-Throughput Transcription-mediated amplification on the Hologic Panther is a highly sensitive method of detection for SARS-CoV-2. J Clin Virol 2020;129(June 2020):104501.
14. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25(3):2000045.
15. Centers for Disease Control and Prevention, CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Available at: https://www.fda.gov/media/134922/download. 2020. Accessed June 23, 2021
16. CerTest BioTec. SARS-CoV-2 (N1 + N2) for BD MAXTM System Instructions for Use. Available at: https://www.certest.es/products/sars-cov-2-n1-n2-bd-maxtm-system/. 2021. Accessed June 23, 2021.
17. Becton Dickinson & Company. SARS-CoV-2 Reagents for BD MAX System Instructions for Use. Available at: https://www.fda.gov/media/136816/download. 2020. Accessed June 23, 2021.
18. Freire-Paspuel B, Vega-Mariño P, Velez A, et al. Analytical and clinical comparison of Viasure (CerTest Biotec) and 2019-nCoV CDC (IDT) RT-qPCR kits for SARS-CoV2 diagnosis. Virology 2021;553:154–6.
19. European Commission. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in Vitro Diagnostic Medical Devices. Vol 75. Available at: https://www.legislation.gov.uk/eudr/1998/79/pdfs/eudr_19980079_1998-12-07_en.pdf. 2003. Accessed June 23, 2021.
20. Stralin M. In which countries is CE marking required? Clever Compliance. Available at: https://support.ce-check.eu/hc/en-us/articles/360014076911-In-which-countries-is-CE-marking-required. 2020. Accessed June 2, 2021
21. European Commission. Q&A on in vitro diagnostic medical device conformity assessment and performance in the context of COVID-19. Available at: https://ec.europa.eu/health/system/files/2021-06/covid-19_ivd-qa_en_0.pdf. 2021. Accessed June 23, 2021
22. Medicines and healthcare products regulatory agency. Regulating medical devices in the UK. 2020. Available at: https://www.gov.uk/guidance/regulating-medical-devices-in-the-uk. Accessed June 28, 2021.
23. European Commission. Regulation (EU) 2017/746 of the European parliament and of the council on in vitro diagnostic medical devices. Off J Eur Union 2017;5(5):117–76.
24. MedTech Europe. Is the IVD Regulation Framework Ready for Class D Devices? Available at: https://www.medtecheurope.org/wp-content/uploads/2020/10/

medtech-europe-reflection-paper-class-d-infrastructure-under-ivdr-transition-october-2020-1.pdf. 2020. Accessed June 23, 2021
25. Mishra S. FDA, CE mark or something else?—thinking fast and slow. Indian Heart J 2017;69(1):1–5.
26. Department of Health and Social care. Private COVID-19 testing validation. 2021. Available at: https://www.gov.uk/government/consultations/private-coronavirus-covid-19-testing-validation/private-covid-19-testing-validation. Accessed June 28, 2021.
27. European Commission. Current performance of COVID-19 test methods and devices and proposed performance criteria. Available at: https://ec.europa.eu/docsroom/documents/40805. 2020. Accessed June 23, 2021
28. European Commission. European commission COVID-19 in vitro diagnostic devices and test methods database. 2020. Available at: https://covid-19-diagnostics.jrc.ec.europa.eu. Accessed June 28.
29. World Health Organization. COVID-19 target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.1.0. World Health Organization; 2020.
30. Medicines and Healthcare products Regulatory Agency. Target product profile: point of care SARS-CoV-2 detection tests 2020. https://doi.org/10.1093/med/9780199609147.003.0077.
31. Medicines and Healthcare products Regulatory Agency. Target product profile: laboratory-based SARS-CoV-2 viral detection tests 2020. https://doi.org/10.1093/med/9780199609147.003.0077.
32. Medicines and Healthcare products Regulatory Agency. Guidance For industry and manufacturers: COVID-19 tests and testing kits. Available at: https://www.gov.uk/government/publications/how-tests-and-testing-kits-for-coronavirus-covid-19-work/for-industry-and-manufactures-covid-19-tests-and-testing-kits. 2021. Accessed June 23, 2021
33. European Centre for Disease Prevention and Control. COVID-19 testing strategies and objectives key messages. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/TestingStrategy_Objective-Sept-2020.pdf. 2020. Accessed June 23, 2021
34. Royal College of Pathologists, COVID-19 testing, 2020, A National Strategy. Available at: https://www.rcpath.org/profession/on-the-agenda/covid-19-testing-a-national-strategy.html. 2020. Accessed June 23, 2021.
35. Torjesen I. Covid-19: how the UK is using lateral flow tests in the pandemic. BMJ 2021;372:1–3.
36. UK Parliament. Mass asymptomatic Covid-19 testing: strategy and accuracy. 2021. Available at: https://commonslibrary.parliament.uk/research-briefings/cbp-9223/. Accessed June 28, 2021.
37. Public Health England. Investigation of novel SARS-COV-2 variant Variant of Concern 202012/01 Detection of an epidemiological cluster associated with a new variant of concern Nomenclature of variants in the UK Current epidemiological findings. 2020;(December):1-11.
38. MobiDiag. Complete solution for rapid molecular diagnostics of coronavirus infection. Available at: https://mobidiag.com/products/coronavirus/#Novodiag-COVID-19. 2021. Accessed June 14, 2021.
39. Abacus Diagnostica. GenomEra SARS-CoV-2 Assay Kit Package Insert. Available at: https://www.abacusdiagnostica.com/products/sars-cov-2-2-0/. 2020. Accessed June 23, 2021.

40. Abacus Diagnostica. GenomEra SARS-CoV-2 , Flu A/B + RSV Assay Kit Package Insert. Version 1.0. Available at: https://www.abacusdiagnostica.com/products/sars-cov-2-flu-a-b-rsv/. 2020. Accessed June 23, 2021
41. BioFire respiratory Panel 2.1 plus (RP2.1plus) Instructions for use. Available at: https://www.biomerieux-diagnostics.com/filmarrayr-respiratory-panel. 2020. Accessed June 23, 2021.
42. GenMark Dx. EPlex SARS-CoV-2 Test Assay Manual. Available at: https://www.fda.gov/media/136282/download. 2020. Accessed June 28, 2021.
43. GenMark Dx. EPlex Respiratory Pathogen Panel 2 Package Insert. Available at: https://www.fda.gov/media/142905/download. 2020. Accessed June 28, 2021.
44. Cepheid. Xpert Xpress SARS-CoV-2/Flu/RSV Instructions For Use. Available at: https://www.cepheid.com/en/package-inserts/1913. 2021. Accessed June 28, 2021.
45. Fournier PE, Zandotti C, Ninove L, et al. Contribution of VitaPCR SARS-CoV-2 to the emergency diagnosis of COVID-19. J Clin Virol 2020;133(January):337–9.
46. Qiagen. QIAstat-Dx Respiratory Panel. Instructions for Use. Available at: https://www.qiagen.com/us/resources/download.aspx?id=e3f2dc10-c712-4bcf-9acd-211bd35df944&lang=en. 2020. Accessed June 28, 2021.
47. Cepheid. Xpert Xpress SARS-CoV-2 Instructions For Use. Available at: https://www.cepheid.com/en/package-inserts/1615. 2020. Accessed June 28, 2021.
48. Lee J, Song JU. Diagnostic accuracy of the Cepheid Xpert Xpress and the Abbott ID NOW assay for rapid detection of SARS-CoV-2: a systematic review and meta-analysis. J Med Virol 2021;(March):1–9.
49. Fitoussi F, Dupont R, Tonen-Wolyec S, et al. Performances of the VitaPCR™ SARS-CoV-2 Assay during the second wave of the COVID-19 epidemic in France. J Med Virol 2021;(March):1–7.
50. Eckbo EJ, Locher K, Caza M, et al. Evaluation of the BioFire COVID-19 test and Respiratory Panel 2.1 for rapid identification of SARS-CoV-2 in nasopharyngeal swab samples. Diagn Microbiol Infect Dis 2021;99(3):115260.
51. Creager HM, Cabrera B, Schnaubelt A, et al. Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2. J Clin Virol 2020 Aug;129:104538. https://doi.org/10.1016/j.jcv.2020.104538.
52. Visseaux B, Le Hingrat Q, Collin G, et al. Evaluation of the QIAstat-dx respiratory SARS-CoV-2 Panel, the first rapid multiplex PCR commercial assay for SARS-CoV- 2 detection. J Clin Microbiol 2020;58(8):1–5.
53. Luminex. ARIES SARS-CoV-2 Assay Package Insert. Available at: https://www.fda.gov/media/136693/download. 2020. Accessed June 28, 2021.
54. MobiDiag. Novodiag COVID-19 Instructions For Use. Document Version 6-0. 2020.
55. Magleby R, Westblade LF, Trzebucki A, et al. Impact of severe acute respiratory syndrome Coronavirus 2 Viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. Clin Infect Dis 2020.
56. Visseaux B, Le Q, Collin G, et al. Evaluation of the RealStar® SARS-CoV-2 RT-PCR kit RUO performances and limit of detection. J Clin Virol 2020;129:104520.
57. Public Health England. Rapid Assessment of the genetic signatures Easy-Screen SARS -CoV-2 detection kit. Available at: https://www.gov.uk/government/publications/covid-19-phe-laboratory-assessments-of-molecular-tests. 2020. Accessed June 23, 2021.
58. Freire-Paspuel B, Garcia-Bereguiain MA. Analytical sensitivity and clinical performance of a triplex RT-qPCR assay using CDC N1, N2, and RP targets for SARS-CoV-2 diagnosis. Int J Infect Dis 2021;102:14–6.

59. Kenyeres B, Anosi N, Banyai K, et al. Comparison of four PCR and two point of care assays used in the laboratory detection of SARS-CoV-2. J Virol Methods 2021;293.
60. Görzer I, Buchta C, Chiba P, et al. First results of a national external quality assessment scheme for the detection of SARS-CoV-2 genome sequences. J Clin Virol 2020;129. https://doi.org/10.1016/j.jcv.2020.104537.
61. Labbé AC, Benoit P, Gobeille Paré S, et al. Comparison of saliva with oral and nasopharyngeal swabs for SARS-CoV-2 detection on various commercial and laboratory-developed assays. J Med Virol 2021;1–6. https://doi.org/10.1002/jmv.27026.
62. Garg A, Ghoshal U, Patel SS, et al. Evaluation of seven commercial RT-PCR kits for COVID-19 testing in pooled clinical specimens. J Med Virol 2021;93(4):2281–6.
63. Price TK, Bowland BC, Chandrasekaran S, et al. Performance characteristics of severe acute respiratory syndrome coronavirus 2 RT-PCR tests in a single health system. J Mol Diagn 2021;23(2):159–63.
64. Cuong HQ, Hai ND, Linh HT, et al. Comparison of primer-probe sets among different master mixes for laboratory screening of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Biomed Res Int 2020;2020. https://doi.org/10.1155/2020/7610678.
65. Procop GW, Brock JE, Reineks EZ, et al. A comparison of five SARS-CoV-2 molecular assays with clinical correlations. Am J Clin Pathol 2021;155(1):69–78.
66. Wirden M, Feghoul L, Bertine M, et al. Multicenter comparison of the Cobas 6800 system with the RealStar RT-PCR kit for the detection of SARS-CoV-2. J Clin Virol 2020;130.
67. Kohmer N, Rabenau HF, Hoehl S, et al. Comparative analysis of point-of-care, high-throughput and laboratory-developed SARS-CoV-2 nucleic acid amplification tests (NATs). J Virol Methods 2021;291. https://doi.org/10.1016/j.jviromet.2021.114102.
68. Lee CK, Tham JWM, Png S, et al. Clinical performance of Roche xobas 6800, Luminex ARIES, MiRXES fortitude kit 2.1, altona RealStar, and applied Biosystems TaqPath for SARS-CoV-2 detection in nasopharyngeal swabs. J Med Virol 2021;1–5. https://doi.org/10.1002/jmv.26940.
69. van Kasteren PB, van der Veer B, van den Brink S, et al. Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. J Clin Virol 2020;128.
70. Merindol N, Pépin G, Marchand C, et al. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. J Clin Virol 2020;128:3–6.
71. Schnuriger A, Perrier M, Marinho V, et al. Caution in interpretation of SARS-CoV-2 quantification based on RT-PCR cycle threshold value. Diagn Microbiol Infect Dis 2021;100(3):2–5. https://doi.org/10.1016/j.diagmicrobio.2021.115366.
72. Public Health England. Rapid assessment of biomerieux real-time detection kit. Available at: https://www.gov.uk/government/publications/covid-19-phe-laboratory-assessments-of-molecular-tests. 2020. Accessed June 23, 2021.
73. Tastanova A, Stoffel CI, Dzung A, et al. A comparative study of real-time RT-PCR–based SARS-CoV-2 detection methods and its application to human-derived and surface swabbed material. J Mol Diagn 2021;23(7):796–804.
74. A.Menarini Diagnostics. CORONAMELT; 2020. Available at: https://www.menarinidiagnostics.com/en-us/Home/Laboratory-products/COVID-19/Viral-RNA-Detection/CoronaMelt/Overview. Accessed June 28, 2021.

75. Altona Diagnostics. The altona Diagnostics product portfolio. 2021. Available at: https://altona-diagnostics.com/en/the-altona-product-lines.html. Accessed June 28, 2021.
76. CerTest BioTec. VIASURE real time PCR detection kits SARS-CoV-2, FLU & RSV. Available at: https://www.certest.es/products/sars-cov-2-flu-rsv/, 2021. Accessed June 28, 2021.
77. Thermo Fisher scientific. TaqManTM SARS-CoV-2, Flu A/B, RSV RT-PCR assay kit. 2021. Available at: https://www.thermofisher.com/order/catalog/product/A47702#/A47702https://www.thermofisher.com/order/catalog/product/A47702#/A47702. Accessed June 28, 2021.
78. Vela diagnostics. ViroKey SARS-CoV-2 RT-PCR test v2.0 (CE-IVD). 2021. Available at. https://www.veladx.com/product/qpcr-respiratory-infections/virokey-sars-cov-2-rt-pcr-test-v20-ce-ivd.html. Accessed June 29, 2021.
79. Lima A, Healer V, Vendrone E, et al. Validation of a modified CDC assay and performance comparison with the NeuMoDxTM and DiaSorin® automated assays for rapid detection of SARS-CoV-2 in respiratory specimens. J Clin Virol 2020 Dec;133:104688. https://doi.org/10.1016/j.jcv.2020.104688.
80. Navarathna DH, Sharp S, Lukey J, et al. Understanding false positives and the detection of SARS-CoV-2 using the Cepheid Xpert Xpress SARS-CoV-2 and BD MAX SARS-CoV-2 assays. Diagn Microbiol Infect Dis 2021;100(1). https://doi.org/10.1016/j.diagmicrobio.2021.115334.
81. EliTech Group. SARS-CoV-2 PLUS ELITe MGB kit. 2020. Available at. https://www.elitechgroup.com/product/sars-cov-2-plus-elite-mgb-kit. Accessed June 28, 2021.
82. Aus Diagnostics. SARS-COV-2, INFLUENZA AND RSV 8-WELL Instructions for Use. 2021.
83. Mannonen L, Kallio-Kokko H, Loginov R, et al. Comparison of two commercial platforms and a laboratory-developed test for detection of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) RNA. J Mol Diagn 2021;23(4):407–16.
84. Mostafa HH, Hardick J, Morehead E, et al. Comparison of the analytical sensitivity of seven commonly used commercial SARS-CoV-2 automated molecular assays. J Clin Virol 2020;130. https://doi.org/10.1016/j.jcv.2020.104578.
85. Aretzweiler G, Leuchter S, Simon CO, et al. Generating timely molecular diagnostic test results: workflow comparison of the cobas® 6800/8800 to Panther. Expert Rev Mol Diagn 2019;19(10):951–7.
86. Khan KA, Cheung P. Presence of mismatches between diagnostic PCR assays and coronavirus SARS-CoV-2 genome: sequence mismatches in SARS-CoV-2 PCR. R Soc Open Sci 2020;7(6):200636.
87. Craney AR, Velu PD, Satlin MJ, et al. Comparison of two high-throughput reverse transcription-PCR systems for the detection of severe acute respiratory syndrome coronavirus 2. J Clin Microbiol 2020;58(8):1–6.
88. Lieberman J, Pepper G, Naccache S, et al. Comparison of commercially available and laboratory developed assays for in vitro detection of SARS-CoV-2 in clinical laboratories. J Clin Microbiol 2020;58(8):e00821-20.
89. Kogoj R, Kmetič P, Valenčak AO, et al. Real-life head-to-head comparison of performance of two high-throughput automated assays for detection of SARS-CoV-2 RNA in nasopharyngeal swabs: the Alinity m SARS-CoV-2 and cobas 6800 SARS-CoV-2 assays. J Mol Diagn 2021. https://doi.org/10.1016/j.jmoldx.2021.05.003.

90. Perchetti GA, Pepper G, Shrestha L, et al. Performance characteristics of the Abbott Alinity m SARS-CoV-2 assay. J Clin Virol 2021;140(May):104869.
91. Smith E, Zhen W, Manji R, et al. Analytical and clinical comparison of three nucleic acid amplification tests for SARS-CoV-2 detection. J Clin Microbiol 2020; 58(9):e01134-20.
92. Fung B, Gopez A, Servellita V, et al. Direct comparison of SARS-CoV-2 analytical limits of detection across seven molecular assays. J Clin Microbiol 2020;58(9): e01535-20.
93. Baker RE, Park SW, Yang W, et al. The impact of COVID-19 nonpharmaceutical interventions on the future dynamics of endemic infections. Proc Natl Acad Sci U S A 2020;117(48):30547–53.
94. Cheng A, Riedel S, Arnaout R, et al. Verification of the Abbott Alinity m Resp-4-Plex Assay for detection of SARS-CoV-2, influenza A/B, and respiratory syncytial virus. medRxiv 2021.
95. Cepheid. GeneXpert-Infinity. 2021. Available at: https://www.cepheid.com/en_US/systems/GeneXpert-Family-of-Systems/GeneXpert-Infinity. Accessed July 1, 2021.
96. Shahhosseini N, Babuadze G, Wong G, et al. Mutation signatures and in silico docking of novel sars-cov-2 variants of concern. Microorganisms 2021;9(5): 1–15. https://doi.org/10.3390/microorganisms9050926.
97. Davies NG, Abbott S, Barnard R, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. Science 2021;372(6538).
98. Tegally H, Wilkinson E, Giovanetti M, et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv 2020;2. https://doi.org/10.1101/2020.12.21.20248640.
99. Andreano E, Piccini G, Licastro D, et al. SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. bioRxiv 2020. https://doi.org/10.1101/2020.12.28.424451.
100. Greaney AJ, Loes AN, Crawford KHD, et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe 2021;29(3):463–76.e6.
101. Ramanathan K, Antognini D, Combes A, et al. Comprehensive mapping of mutations in the SARS CoV- 2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe 2020;(January):19–21.
102. Weisblum Y, Schmidt F, Zhang F, et al. Escape from neutralizing antibodies 1 by SARS-CoV-2 spike protein variants. Elife 2020;9:1.
103. Rambaut A, Loman N, Pybus O, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. Available at: https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563. 2021. Accessed June 23, 2021.
104. Sabino EC, Buss LF, Carvalho MPS, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. Lancet 2021;397(10273):452–5.
105. Yi C, Sun X, Ye J, et al. Key residues of the receptor binding motif in the spike protein of SARS-CoV-2 that interact with ACE2 and neutralizing antibodies. Cell Mol Immunol 2020;17(6):621–30.
106. Cagliani R, Forni D, Clerici M, et al. Computational inference of selection underlying the evolution of the novel coronavirus, severe acute respiratory syndrome coronavirus 2. J Virol 2020;94(12):e00411-20.

107. Ou J, Zhou Z, Dai R, et al. Emergence of rbd mutations in circulating sars-cov-2 strains enhancing the structural stability and human ace2 receptor affinity of the spike protein. bioRxiv 2020;1–30.
108. World Health Organization. Tracking SARS-CoV-2 variants. 2021. Available at: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/. Accessed June 28, 2021.
109. Thermo Fisher scientific. TaqMan SARS-CoV-2 mutation Panel. 2021. Available at: https://www.thermofisher.com/uk/en/home/clinical/clinical-genomics/pathogen-detection-solutions/real-time-pcr-research-solutions-sars-cov-2/mutation-panel.html. Accessed June 29, 2021.
110. Bioscience A. MassARRAY® SARS-CoV-2 variant Panel (RUO). 2021. Available at: https://www.agenabio.com/wp-content/uploads/2021/03/GEN0048-05-SC2-Variant-Product-Sheet.pdf. Accessed July 1, 2021.
111. Kakhki RK, Kakhki MK, Neshani A. COVID-19 target: a specific target for novel coronavirus detection. Gene Rep 2020;20:100740.
112. Wu D, Koganti R, Lambe UP, et al. Vaccines and therapies in development for SARS-CoV-2 infections. J Clin Med 2020;9(6):1885.
113. Kim C-H. SARS-CoV-2 evolutionary adaptation toward host entry and recognition of receptor O-acetyl sialylation in virus–host interaction. Int J Mol Sci 2020;21(12):4549.
114. Huang C, Lokugamage KG, Rozovics JM, et al. SARS coronavirus nsp1 protein induces template-dependent endonucleolytic cleavage of mRNAs: viral mRNAs are resistant to nsp1-induced RNA cleavage. PLOS Pathog 2011;7(12):e1002433.
115. Cornillez-Ty CT, Liao L, Yates JR 3rd, et al. Severe acute respiratory syndrome coronavirus nonstructural protein 2 interacts with a host protein complex involved in mitochondrial biogenesis and intracellular signaling. J Virol 2009;83(19):10314–8.
116. Korber B, Fischer WM, Gnanakaran S, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 2020;182(4):812–27.e19.
117. Oostra M, Te Lintelo EG, Deijs M, et al. Localization and membrane topology of coronavirus nonstructural protein 4: involvement of the early secretory pathway in replication. J Virol 2007;81(22):12323–36.
118. Chan JF-W, Kok K-H, Zhu Z, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 2020;9(1):221–36.
119. Benvenuto D, Angeletti S, Giovanetti M, et al. Evolutionary analysis of SARS-CoV-2: how mutation of Non-Structural Protein 6 (NSP6) could affect viral autophagy. J Infect 2020;81(1):e24–7.
120. Cottam EM, Whelband MC, Wileman T. Coronavirus NSP6 restricts autophagosome expansion. Autophagy 2014;10(8):1426–41.
121. Wang Y, Sun Y, Wu A, et al. Coronavirus nsp10/nsp16 methyltransferase can Be targeted by nsp10-derived peptide in vitro and in vivo to reduce replication and pathogenesis. J Virol 2015;89(16):8416–27.
122. Suryawanshi RK, Koganti R, Agelidis A, et al. Dysregulation of cell signaling by SARS-CoV-2. Trends Microbiol 2021;29(3):224–37.
123. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579(7798):265–9.

124. Seybert A, Hegyi A, Siddell SG, et al. The human coronavirus 229E superfamily 1 helicase has RNA and DNA duplex-unwinding activities with 5'-to-3' polarity. Rna 2000;6(7):1056–68.
125. Chang C-k, Hou M-H, Chang C-F, et al. The SARS coronavirus nucleocapsid protein – forms and functions. Antiviral Res 2014;103:39–50.
126. Chen Y, Cai H, Pan J, et al. Functional screen reveals SARS coronavirus nonstructural protein nsp14 as a novel cap N7 methyltransferase. Proc Natl Acad Sci 2009;106(9):3484.
127. Naqvi AAT, Fatima K, Mohammad T, et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: structural genomics approach. Biochim Biophys Acta Mol Basis Dis 2020;1866(10):165878.
128. Castaño-Rodriguez C, Honrubia JM, Gutiérrez-Álvarez J, et al. Role of severe acute respiratory syndrome coronavirus viroporins E, 3a, and 8a in replication and pathogenesis. mBio 2018;9(3):e02325-17.
129. Yount B, Roberts RS, Sims AC, et al. Severe acute respiratory syndrome coronavirus group-specific open reading frames encode nonessential functions for replication in cell cultures and mice. J Virol 2005;79(23):14909–22.
130. Xia H, Cao Z, Xie X, et al. Evasion of type I interferon by SARS-CoV-2. Cell Rep 2020;33(1):108234.
131. Chen S, Zheng X, Zhu J, et al. Extended ORF8 gene region is valuable in the epidemiological investigation of severe acute respiratory syndrome–similar coronavirus. J Infect Dis 2020;222(2):223–33.
132. Wang M-Y, Zhao R, Gao L-J, et al. SARS-CoV-2: structure, biology, and structure-based therapeutics development. Front Cell Infect Microbiol 2020; 10:724.
133. MobiDiag. Coronavirus solutions. 2021. Available at: https://mobidiag.com/products/coronavirus/. Accessed June 29, 2021.
134. MobiDiag. Amplidiag COVID-19 Instructions for Use. 2020.
135. Jokela P, Jääskeläinen AE, Jarva H, et al. SARS-CoV-2 sample-to-answer nucleic acid testing in a tertiary care emergency department: evaluation and utility. J Clin Virol 2020;131(January). https://doi.org/10.1016/j.jcv.2020.104614.
136. Aus diagnostics. Highplex Alliance™. 2021. Available at: https://www.ausdiagnostics.com/highplex-alliance. Accessed June 29, 2021.
137. Pham J, Meyer S, Nguyen C, et al. Performance characteristics of a high-throughput automated. J Clin Microbiol 2020;1–6.
138. Schneider M, Iftner T, Ganzenmueller T. Evaluation of the analytical performance and specificity of a SARS-CoV-2 transcription-mediated amplification assay. J Virol Methods 2021;294:114182.
139. Zhen W, Manji R, Smith E, et al. Comparison of four molecular in vitro diagnostic assays for the detection of sars-cov-2 in nasopharyngeal specimens. J Clin Microbiol 2020;58.
140. Wong RCW, Wong AH, Ho YII, et al. Performance evaluation of Panther Fusion SARS-CoV-2 assay for detection of SARS-CoV-2 from deep throat saliva, nasopharyngeal, and lower-respiratory-tract specimens. J Med Virol 2021;93: 1226–8.
141. Dust K, Hedley A, Nichol K, et al. Comparison of commercial assays and laboratory developed tests for detection of SARS-CoV-2. J Virol Methods 2020;285.
142. EliTech Group. SARS-CoV-2 variants ELITe MGB® kit. Available at: https://www.elitechgroup.com/product/sars-cov-2-variants-elite-mgb-kit. Accessed June 29, 2021.

143. CerTest BioTec. SARS-CoV-2 & UK variant (S UK, ORF1ab and N genes). 2021. Available at: https://www.certest.es/products/sars-cov-2-uk-variant-s-uk-orf1ab-and-n-genes/. Accessed June 29, 2021.
144. Antolia geneworks. Bosphore SARS-CoV-2 variant detection kit v1. 2021. Available at: http://www.anatoliageneworks.com/en/kitler.asp?id=375&baslik=Bosphore SARS-CoV-2 Variant Detection Kit v1&bas=Bosphore SARS-CoV-2 Variant Detection Kit v1. Accessed June 29, 2021.
145. TIB MOLBIOL. SARS kits and VirSNiP assays. 2021. Available at: https://www.tib-molbiol.de/covid-19. Accessed June 29, 2021.