SARS-CoV-2 Variant Panel (RUO):

A high throughput and robust assay for the detection and differentiation of key SARS-CoV-2 variants of concern for use on the MassARRAY System

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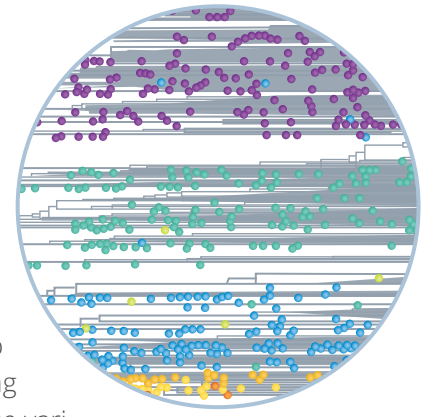


ABSTRACT

Agena Bioscience® has developed a Research Use Only (RUO) panel for the detection and differentiation of SARS-CoV-2 variants on the MassARRAY® System. This panel provides a robust alternative to next-generation sequencing (NGS) to screen for the presence of SARS-CoV-2 variants of concern (VOCs) in human samples. This two-well panel utilizes a one-step RT-PCR reaction to reverse transcribe viral RNA into cDNA and amplify the nucleic acid material in the same reaction. The high multiplexing capabilities of the iPLEX® Pro chemistry allow for the simultaneous detection of unique variants in this first version of the panel, with the capability to include additional VOCs quickly and easily as they emerge. Currently, the panel differentiates the B.1.1.7 (UK), B.1.351 (South Africa), B.1.1.248 / P.1 (Brazil), Cluster 5/Mink (Denmark) and D614G variants from the A.1 (Wuhan) lineage. The high-throughput MassARRAY System enables laboratories to cost-effectively process from hundreds up to thousands of samples per day with a single instrument, without the need for extensive bioinformatics analysis or infrastructure.

THE NEED FOR SARS-CoV-2 VARIANT SCREENING

Over the course of the COVID-19 pandemic new genetic variants of the etiologic agent, SARS-CoV-2, have spread widely across the globe. Genetic changes arise as a product of the replication of the virus' RNA genome. It is well recognized that RNA viruses have a higher mutation rate than DNA viruses. RNA replication for the SARS-CoV-2 virus requires several viral encoded proteins, including the RNA-dependent RNA polymerase, referred to as non-structural protein 12 (Nsp12).[1] Most relevant to this discussion is the enzyme NSP14, which is part of a proofreading system responsible for the high fidelity of SARS-CoV-2 replication.[2] Because of this error correcting function, coronaviruses, including SARS-CoV-2, generate fewer mutations over time than do many other RNA genome viruses. However, this system is not perfect, and several genetic variants have been identified that give those altered SARS-CoV-2 genetic variants a competitive advantage over their unaffected cousins. Such genetic changes have been shown to result in the following phenotypes:

- Stronger binding to viral receptors (the ACE2 protein[3]) on the surface of respiratory tract cells
- Enhanced entry into the target cell[3]
- Increased production of the virus spike protein within infected cells[4]
- Conformational changes in the virus spike protein to help evade host immune response[5]
- Mutations in the furin cleavage site of the spike protein to modulate its pathogenicity[17]

The Agena Bioscience MassARRAY SARS-CoV-2 Panel is for *in vitro* diagnostic use.
All other products are for research use only. Not for use in diagnostic procedures.

The United States Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) have established a classification scheme for variants of SARS-CoV-2. These classifications include definitions and attributes of the variants:

- Variants of interest
- Variants of concern
- Variants of high consequence

The definition of a **variant of interest** is "a variant with specific genetic markers that have been associated with changes to receptor binding, reduced neutralization by antibodies generated against previous infection or vaccination, reduced efficacy of treatments, potential diagnostic impact, or predicted increase in transmissibility or disease severity". The list of such variants as of March 2021 is shown in Table 1.

Table 1: Variants of Interest[20]

Name (Pango lineage)	Genetic Markers	Name (Nextstrain)	First Detected	Predicted Attributes
B.1.526	**Spike:** (L5F*), T95I, D253G, (S477N*), (E484K*), D614G, (A701V*)	20C	New York November 2020	Potential reduction in neutralization by monoclonal antibody treatments
	ORF1a: L3201P, T265I, Δ3675/3677			Potential reduction in neutralization by convalescent and post-vaccination sera
	ORF1b: P314L, Q1011H			
	ORF3a: P42L, Q57H			
	ORF8: T11I			
	5'UTR: R81C			
B.1.525	**Spike:** A67V, Δ69/70, Δ144, E484K, D614G, Q677H, F888L	20C	New York November 2020	Potential reduction in neutralization by monoclonal antibody treatments
	ORF1b: P314F			Potential reduction in neutralization by convalescent and post-vaccination sera
	ORF1a: T2007I			
	M: I82T			
	N: A12G, T205I			
	5'UTR: R81C			
P.2	**Spike:** E484K, D614G, V1176F	20J	Brazil April 2020	Potential reduction in neutralization by monoclonal antibody treatments
	ORF1a: L3468V, L3930F			Potential reduction in neutralization by convalescent and post-vaccination sera
	ORF1b: P314L			
	N: A119S, R203K, G204R, M234I			
	5'UTR: R81C			

*Abbreviations: **N** = nucleocapsid protein; **ORF** = open reading frame; 5`UTR – 5` untranslated region.*

Variants of interest may display the following attributes:

- Specific genetic markers predicted to affect transmission, diagnostics, therapeutics, or immune escape
- Evidence demonstrating it causes an increased proportion of cases or unique outbreak clusters
- Limited prevalence or expansion in the US or in other countries

The definition of a **variant of concern** is "a variant for which there is evidence of an increase in transmissibility, more severe disease (increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures". The specific attributes of a variant of concern may include:

- Evidence of impact on diagnostics, treatments, and vaccines
    - Widespread interference with diagnostic test targets
    - Evidence of substantially increased resistance to one or more classes of therapies
    - Evidence of significant decreased neutralization by antibodies generated during previous infection or vaccination
    - Evidence of reduced vaccine-induced protection from severe disease
- Evidence of increased transmissibility
- Evidence of increased disease severity

Based on the characteristics of the variant, additional considerations may include the development of new diagnostics or the modification of vaccines or treatments. The current list of variants of concern is shown in Table 2.

Table 2: Variants of Concern[20]

Name (Pango lineage)	Spike Protein Genetic Markers	Name (Nextstrain)	First Detected	Predicted Attributes
B.1.1.7	Δ6970, Δ144Y, (E484K*), (S494P*), N501Y, A570D, D614G, P681H	20I/501Y.V1	United Kingdom 2020	~50% increased transmission; Likely increased severity based on hospitalizations and case fatality rates; Minimal impact on neutralization by EUA monoclonal antibody therapeutics; Minimal impact on neutralization by convalescent and post-vaccination sera
P.1	K417N/T, E484K, N501Y, D614G	20J/501Y.V3	Japan / Brazil 2020	Moderate impact on neutralization by EUA monoclonal antibody therapeutics; Reduced neutralization by convalescent and post-vaccination sera
B.1.351	K417N, E494K, N501Y, D614G	20H/501.V2	South Africa 2020	~50% increase in transmission; Moderate impact on neutralization by EUA monoclonal antibody therapeutics; Moderate reduction in neutralization by convalescent and post-vaccination sera
B.1.427	L452R, D614G	20C/S:452R	USA-California 2021	~20% increased transmissibility; Significant impact on neutralization by some, but not all, EUA therapeutics; Moderate reduction in neutralization using convalescent and post-vaccination sera
B.1.429	L452R, D614G, W152C, S13I	20C/S:452R	USA-California 2021	~20% increased transmissibility; Significant impact on neutralization by some, but not all, EUA therapeutics; Moderate reduction in neutralization using convalescent and post-vaccination sera

These variants of interest and variants of concern all share one specific genetic marker, D614G. This genetic marker was one of the first documented in the US in the initial stages of the pandemic, after having initially circulated in Europe.[18] There is evidence that variants with this genetic marker spread more quickly than viruses without.[19]

The third variant category is called **variants of high consequence**. Variants that fall under this category have "clear evidence that prevention measures or medical countermeasures have significantly reduced effectiveness relative to previously circulating variants". Possible attributes of this class of variants include:

- Demonstrated failure of diagnostics
- Evidence to suggest a significant reduction in vaccine effectiveness, a disproportionately high number of vaccine breakthrough cases, or very low vaccine-induced protection against severe disease
- Significantly reduced susceptibility to multiple Emergency Use Authorization (EUA) or approved therapeutics
- More severe clinical disease and increased hospitalizations

A variant of high consequence would require public health officials to declare a public health emergency of international concern (PHEIC - if not already declared), reporting to the Centers for Disease Prevention and Control, make an announcement of strategies to prevent or contain transmission, and make recommendations to update treatments and vaccines.

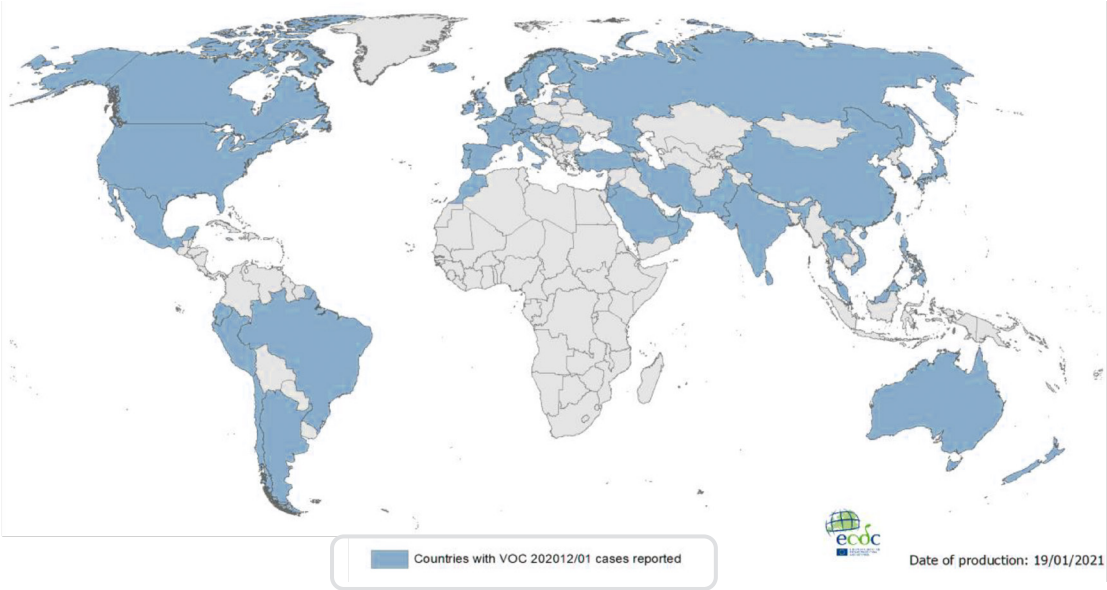
Resources for following the spread of variants of concern:

EU - ECDC Report "Risk related to the spread of new SARS-CoV-2 variants of concern in the EU/EEA – first update" https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-risk-related-to-spread-of-new-SARS-CoV-2-variants-EU-EEA-first-update.pdf

US - Interactive website, "US COVID-19 Cases Caused by Variants", which is regularly updated: https://www.cdc.gov/coronavirus/2019-ncov/transmission/variant-cases.html

An example of the information available at is shown in **Figure 1**.

Figure 1: *Countries reporting cases of VOC 2020 12/01 worldwide, as of 19 January 2021*



The CDC website has an interactive website showing global spread of specific variants: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/global-variant-map.html

The SARS-CoV-2 variant designated B.1.1.7 that originated in the United Kingdom (UK) has been detected around the world. This variant carries multiple genetic markers in the spike protein, relative to the original Wuhan strain, that are associated with enhanced transmissibility (**Table 2**). The spike protein 69-70 deletion is one of the most common spike protein deletion mutations, resulting in altered antigenicity, leading to resistance against neutralizing antibodies. Two additional variants, B.1.351 and P.1, (referred to in **Table 2**) arose in South Africa and Brazil respectively. These share several of the same spike genetic markers carried by B.1.1.7, and also carry other spike protein genetic markers: E484K, and either K417N (B.1.351) or K417T (P.1). These lead to alterations in the protein receptor binding domain (RBD), resulting in weaker neutralization of these viruses with sera from patients immunized with the Moderna and Pfizer COVID-19 vaccines.[10] Multiple lines of evidence support escape from antibody selective pressure as a driving force for the development of these variants.

It is very clear that surveillance for the spread of current SARS-CoV-2 variants and the emergence of new variants is an important public health function. Such monitoring is necessary to determine the ability of these new viruses to:

- Spread more quickly
- Cause either milder or more severe disease
- Evade detection by specific diagnostic tests
- Decrease susceptibility to therapeutic agents such as monoclonal antibodies
- Evade natural or vaccine-induced immunity

To date, such surveillance efforts have been performed by multiple institutions like the Centers for Disease Control and Prevention (CDC) in the US, Covid-19 Genomics UK Consortium (COG-UK), Deutscher Elektronischer Sequenzdaten-Hub (DESK) in Germany, state public health laboratories, academic institutions, and laboratories. The method predominately used has been next generation sequencing. This is a powerful tool that allows for the very sensitive detection of new mutations and variants of interest that are emerging and spreading throughout our communities.

The sequence data are mainly deposited into one (or both) of the following databases: National Center for Biotechnology Information (NCBI) or Global Initiative on Sharing Avian Influenza Data (GISAID).

These data have allowed the detection of the origin of the variants currently circulating worldwide. Several variants share the same genetic markers; for example, N501Y, which is common to all three of the variants just mentioned. Also, B.1.351 and B.1.1.258 share the E484K genetic marker. E484K has been further identified in two variants, P.2 (Brazil) and B.1.2 (Southern California). Such sharing of identical genetic markers by different variants can occur when a new variant is derived from one that already exists, or through convergent evolutionary mechanisms.

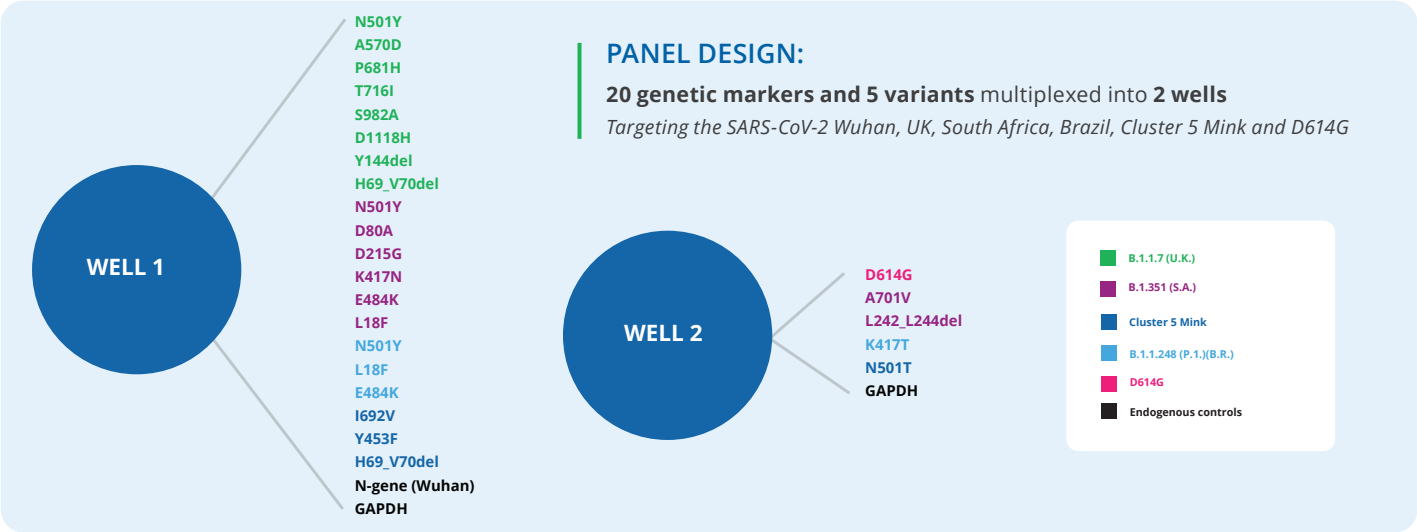
Although NGS is a powerful tool, with its main advantage being the detection of *new and emerging variants of interest,* it may not be the method of choice for following the spread of *known VOCs* throughout the population for very specific reasons. There are relevant downsides that should be considered for NGS, including:

- Expensive to perform
- Slow turn-around time for results
- Low sample throughput
- Requirement for extensive bioinformatics capabilities to analyze results

SARS-COV-2 VARIANT PANEL

To efficiently follow the spread of VOCs a platform must overcome these NGS shortcomings. For this purpose, Agena Bioscience has developed the MassARRAY SARS-CoV-2 Variant Panel, a high throughput, low-cost, and rapid assay for the detection of 20 unique SARS-CoV-2 genetic markers. The panel is available in 96- and 384-well formats, to accommodate different testing volume needs. The assay differentiates the B.1.1.7 (UK), B.1.351 (South Africa), B.1.1.248 / P.1 (Brazil), Cluster 5/Mink (Denmark) and D614G variants from the A.1 (Wuhan) lineage. All genetic markers in this panel are spike protein mutations of concern that also define the particular variants. Variant D614G is included as it is an important VOC, the presence of which is known to enhance transmissibility of the affected virus. The specific variants detected are shown in **Figure 3**.

Figure 3: *MassARRAY SARS-CoV-2 Variant Panel v1 (RUO)*



Assay Design

The panel primers were analyzed *in silico* for sequence specificity to SARS-CoV-2 by performing NCBI BLAST, *in silico* PCR at UCSC Genome Browser, and alignment of panel primers to a multiple sequence alignment of variants intended to be interrogated by the panel. The panel primers showed 100% sequence homology to the intended targets apart from any tags utilized for mass spectrum placement.

NCBI nucleotide BLAST was used to test for cross reactivity of the primers *in silico* using a list of FDA-recommended micro-organisms (see **Appendix A**). The combination of primers for each assay (two PCR primers and one extension probe) exhibit 100% sequence homology to the conserved SARS-CoV-2 regions. All individual primers (one of two PCR primers and/or the extension probes) for each of the assays exhibit lower than the 80% homology to a cross-reactive species. The likelihood of false positive results is extremely low.

ANALYSIS SOFTWARE

Data analysis is simplified using supporting software which qualifies the RNA sample and determines the detection status of the virus. A sample passes QC based on the results of the GAPDH assay, which serves as an internal control for the nucleic acid extraction, reverse transcription (RT), PCR, dephosphorylation (SAP), and extension steps. As the SARS-CoV-2 variant panel is designed as a reflex test for samples that have already tested positive for SARS-CoV-2, the panel also tests for the presence of the SARS-CoV-2 N-gene. Variant calling is performed according to the criteria shown in **Figure 4**. These thresholds for detection can be modified by the laboratory.

Figure 4: *Variant Calling Summary*

B.1.1.7 (U.K.)	B.1.351 (S.A.)	B.1.1.248 (P.1) (B.R.)	Cluster 5 Mink	D614G
• PP681H • N501Y • A570D • H69_V70del • S982A • D1118H • T716I • Y144del_Com	• N501Y • D215G • E484K • D80A • K417N • L242_L244del • A701V • L18F	• N501Y • L18F • E484K • K417T	• I692V • N501T • H59_V70del • Y453F	• D614G
Required Positive: 6/8	6/8	4/4	3/4	1/1

An example of the run report is shown in **Figure 5**. The report includes all relevant information including the sample ID, sample type, plate location, instrument ID, QC status, relevant messages, as well as the variant status and specific genetic markers detected for each sample.

Figure 5: Run Report

MassARRAY SARS-CoV-2 Variant Report

Date Tue Mar 23 12:54:58 2021

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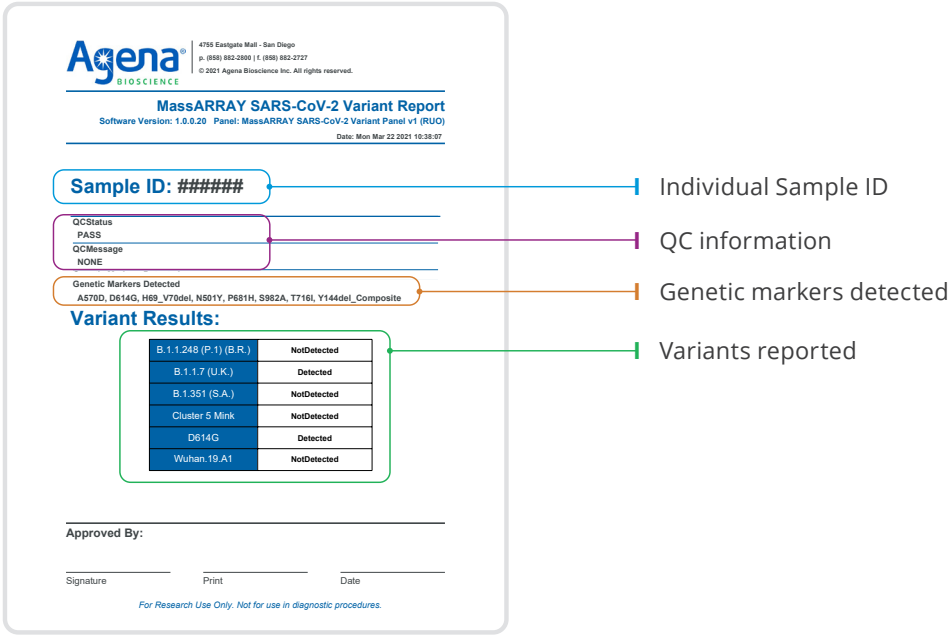
Sample	SampleType	Location	Instrument	QCStatus	QCMessage	Variant Status	Genetic Markers Detected
NC	Negative Control	20210223_	MrBlack-3.4-i	PASS		Control Pass	
PC	Positive Control	20210223_	MrBlack-3.4-i	PASS		Control Pass	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_2	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_2	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_2	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_5	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_5	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_5	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_1	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_1	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_1	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_4	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_4	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_4	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_1	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_1	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_14_1	Test	20210223_	MrBlack-3.4-i	PASS		B.1.1.7 (U.K.) Detected. D614G Detected.	A570D | D614G | H69_V70del | N501Y | P681H
Twist_WT_	Test	20210223_	MrBlack-3.4-i	PASS		Wuhan.19.A1 Detected.	
Twist_WT_	Test	20210223_	MrBlack-3.4-i	WARNING	Not all Endogenous controls detected.	Wuhan.19.A1 Detected.	
Twist_WT_	Test	20210223_	MrBlack-3.4-i	PASS		Wuhan.19.A1 Detected.	
Twist_WT_	Test	20210223_	MrBlack-3.4-i	PASS		Wuhan.19.A1 Detected.	
Twist_WT_	Test	20210223_	MrBlack-3.4-i	PASS		Wuhan.19.A1 Detected.	

A report is also generated for each individual sample. An example of the individual sample report, shown in **Figure 6**, contains all relevant information for each unique sample, including software version, date, sample ID, QC information, genetic markers detected, and the variant reported.

Figure 6: Individual Sample Report

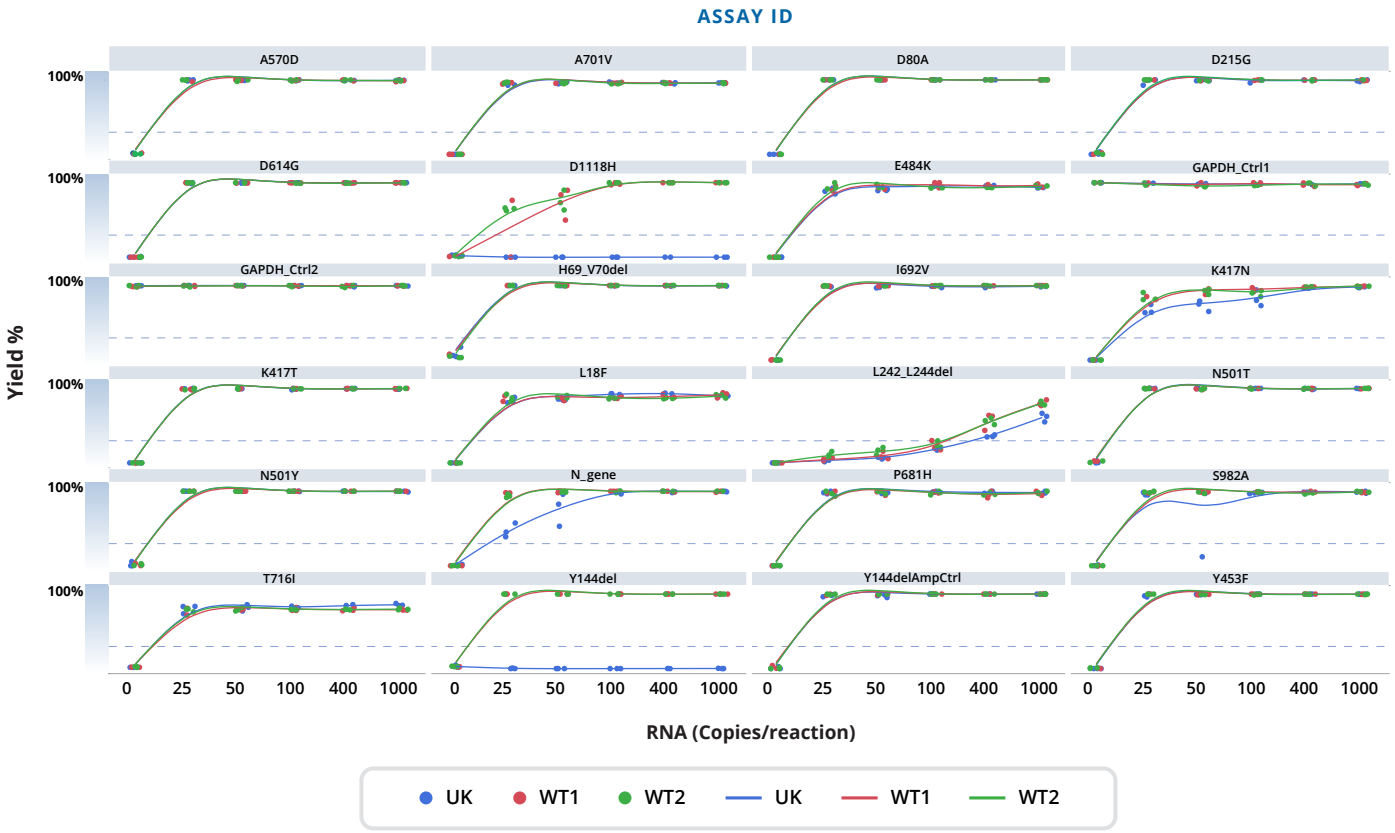


METHODS AND RESULTS

Several experiments were designed to verify the panel performance as described below. Positive samples were generated using SARS-CoV-2 negative human RNA spiked with the Synthetic SARS-CoV-2 RNA Controls from Twist Bioscience (SKU 102019). Clinical samples as well as isolates previously characterized by NGS were also utilized during verification of the panel.

The performance of the assays in detecting each individual variant is shown in **Figure 7**. These are plots of extension rates (yield) versus synthetic SARS-CoV-2 RNA copies input into the reaction in a background of 1 ng human RNA. These results allow the panel to detect and discriminate synthetic SARS-CoV-2 RNAs representative of the UK variant (B.1.1.7) and wild type at 25 copies per reaction input.

Figure 7: *Performance of Individual Assays (Yield vs Copy Number)*



Clinical samples that were pre-screened using either the Abbott RealTime SARS-CoV-2 or Agena MassARRAY SARS-CoV-2 EUA assays were also tested with the MassARRAY SARS-CoV-2 Variant Panel. The results shown in **Table 3** demonstrate that the variant panel had excellent agreement with both EUA assays. There was 1 invalid sample reported with the variant panel within the low-titer SARS-CoV-2 samples due to the endogenous control for the N-gene falling below the detection threshold.

Table 3: Pre-screened Clinical Sample Results

	Ct Value	# of Samples	Abbott	Agena EUA	Agena Variant Panel
Low-titer SARS-CoV-2	> 23	7	7	7	6*
Medium-titer SARS-CoV-2	17 - 23	5	5	5	5
High-titer SARS-CoV-2	< 17	17	17	17	17
Negative	N/A	12	12	12	12

* 1 Internal control failure

In addition to testing clinical samples previously screened with on-market EUA assays, clinical isolates previously sequenced on the Illumina MiSeq with results deposited in GISAID were also analyzed with the MassARRAY SARS-CoV-2 Variant Panel. **Table 4** shows the variants and Ct values for each of the samples.

Table 4: Clinical isolates sequenced on Illumina MiSeq

GISAID Accession	GISAID Virus Name	nCov N1 Ct	nCov N2 Ct	Panther Ct
Lineage B.1.429				
EPI_ISL_737248	hCoV-19/USA/CA-LACPHL-AF00013/2020	N/A	N/A	21.3
EPI_ISL_792675	hCoV-19/USA/CA-LACPHL-AF00085/2020	N/A	N/A	18
Lineage B.1.427				
EPI_ISL_861690	hCoV-19/USA/CA-LACPHL-AG00005/2021	N/A	N/A	16
Lineage B.1.2				
EPI_ISL_833413	hCoV-19/USA/CA-LACPHL-AF00160/2021	13.1	N/A	N/A
Lineage P.2				
EPI_ISL_1097739	hCoV-19/USA/CA-LACPHL-AF00485/2021	15.9	27.5	N/A

Results are shown in the test report (**Figure 8**). All expected variants were correctly identified given the current content within the variant panel.

Figure 8: *MassARRAY SARS-Cov-2 Variant Panel Run Report for Previously Sequenced Clinical Isolates*

MassARRAY SARS-CoV-2 Variant Report

Report version	1.0.0.20						
Panel	MassARRAY SARS-CoV-2 Variant Panel v1 (RUO)						
Date	Mon Mar 8 21:33:38 2021						
For Research Use Only. Not for use in diagnostic procedures.							
Sample	SampleType	Location	Instrument	QCStatus	QCMessage	VariantStatus	Genetic Markers Detected
1097739_P2	Test	20210308_	MA4-3.4-iPLE	PASS		D614G Detected.	D614G | E484K
737248_B1429	Test	20210308_	MA4-3.4-iPLE	PASS		D614G Detected.	D614G
792675_B1429	Test	20210308_	MA4-3.4-iPLE	PASS		D614G Detected.	D614G
833413_B117	Test	20210308_	MA4-3.4-iPLE	PASS		D614G Detected.	D614G | E484K | Y453F
861690_B1427	Test	20210308_	MA4-3.4-iPLE	PASS		D614G Detected.	D614G
PC	Positive Control	20210308_	MA4-3.4-iPLE	PASS		Control Pass	A570D | D614G | H69_V70del | N501Y | P681H | S982A | T716I | Y144del_Composite
NC	Negative Control	20210308_	MA4-3.4-iPLE	PASS		Control Pass	
NC_rep02	Negative Control	20210308_	MA4-3.4-iPLE	PASS		Control Pass	

Below (**Figures 9 – 10**) are examples of individual mass spectra generated from some of the clinical isolates. These are used to highlight examples of the data output for the detection of specific variants.

Figure 9: *Variant B.1.2 (E484K)*

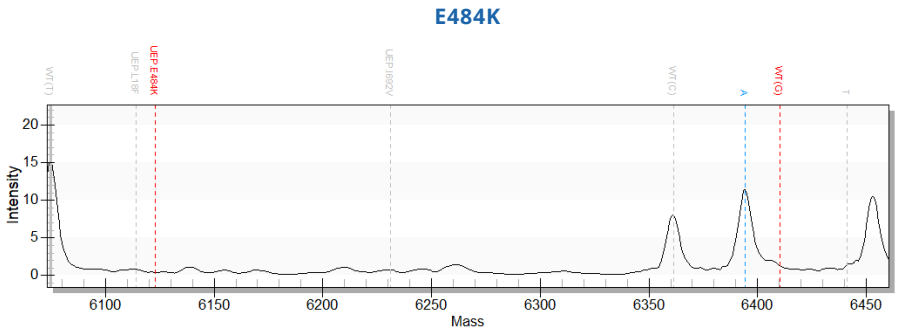
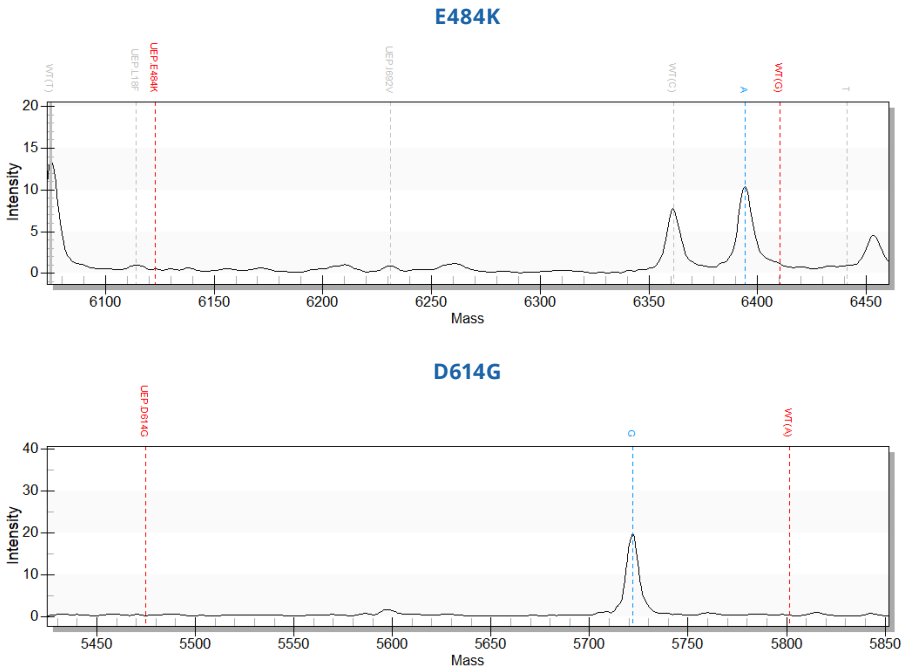


Figure 10: Variant P.1 (E484K and D614G)



CONCLUSIONS

Results of these studies demonstrate that the Agena Bioscience MassARRAY SARS-CoV-2 Variant Panel (RUO) can be used to detect SARS-CoV-2 variants of concern and differentiate key variants. The panel represents an alternative method to NGS for the rapid screening of known variants of concern with the following key advantages:

- **ACCURATE DETECTION** – Identify and differentiate B.1.1.7 (UK), B.1.351 (South Africa), B.1.1.248/P.1 (Brazil), Cluster 5/Mink (Denmark), and D614G from the Wuhan lineage of SARS-CoV-2 with the ability to quickly and easily add new variants of concern as they emerge.
- **MAXIMIZE THROUGHPUT** – Process up to hundreds or thousands of samples on a single instrument in a single workday.
- **SIMPLIFY INTERPRETATION** – Clear identification of variants of concern without the need for extensive bioinformatics analysis.
- **ECONOMICAL** – Low-cost reagents and automated results reporting for under $25 per sample, compared to NGS costs in the range of $25 to $400 depending on the sample batch size.[16]

Additionally, Agena Bioscience is in the process of incorporating additional variants of concern into the panel as they continue to emerge in the US and worldwide.[11,12,13] One such variant, B.1.526, was recently identified in New York City.[14] This variant carries both the E484K and N501Y genetic markers found in B.1.351 from South Africa. Neutralizing activities of convalescent plasma or vaccinee sera were determined to be lower by 7.7-fold or 3.4-fold, respectively, against B.1.526. Another new variant, B.1.429, has also been identified in Southern California.[14] Both the New York and California variants are spreading rapidly within their respective communities. Other spike protein mutations, unrelated to specific variants, are appearing and spreading quickly across the US. Such genetic markers include L425R and Q677P/H, which, like other alterations in the spike protein, increase the infectivity of the virus by increasing the binding affinity to the ACE2 receptor (L425R) or enhancing the efficiency of entry into the cell (Q677P/H).

The continued emergence of SARS-CoV-2 variants worldwide underscores the importance of continued surveillance and research. The MassARRAY workflow offers a sensitive, high-throughput and robust method for the detection and differentiation of SARS-CoV-2 variants of concern.

APPENDIX A: Microorganisms screened for cross-reactivity *(in silico)*

Other high priority pathogens (from the same genetic family)	High priority organisms likely present in a respiratory specimen	
Human coronavirus 229E	Adenovirus (e.g., C1 Ad. 71)	*Bordetella pertussis*
Human coronavirus OC43	Enterovirus (e.g., EV68)	*Candida albicans*
Human coronavirus HKU1	Human Metapneumovirus (hMPV)	*Chlamydia pneumoniae*
Human coronavirus NL63	Influenza A & B	*Haemophilus influenzae*
SARS coronavirus	Parainfluenza virus 1-4	*Legionella pneumophila*
MERS coronavirus	Respiratory syncytial virus	*Mycobacterium tuberculosis*
	Rhinovirus	*Mycoplasma pneumoniae*
		Pneumocystis jirovecii (PJP)
		Pseudomonas aeruginosa
		Staphylococcus epidermis
		Streptococcus pneumoniae
		Streptococcus pyogenes
		Streptococcus salivarius

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The Agena Bioscience MassARRAY SARS-CoV-2 Panel is for *in vitro* diagnostic use.
All other products are for research use only. Not for use in diagnostic procedures.

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