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Analysis of SARS-CoV-2 using LC-MS Peptide Enrichment for Clinical Research

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Outline

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Overview of SARS-CoV-2 and the role of LC-MS/MS in its detection for clinical research

Overview of a Kit-based LC-MS RUO Workflow for detecting SARS-CoV-2 NCAP Peptides

Performance Characteristics of the Kit-based LC-MS RUO Workflow

SARS-CoV-2 virion and it's proteins

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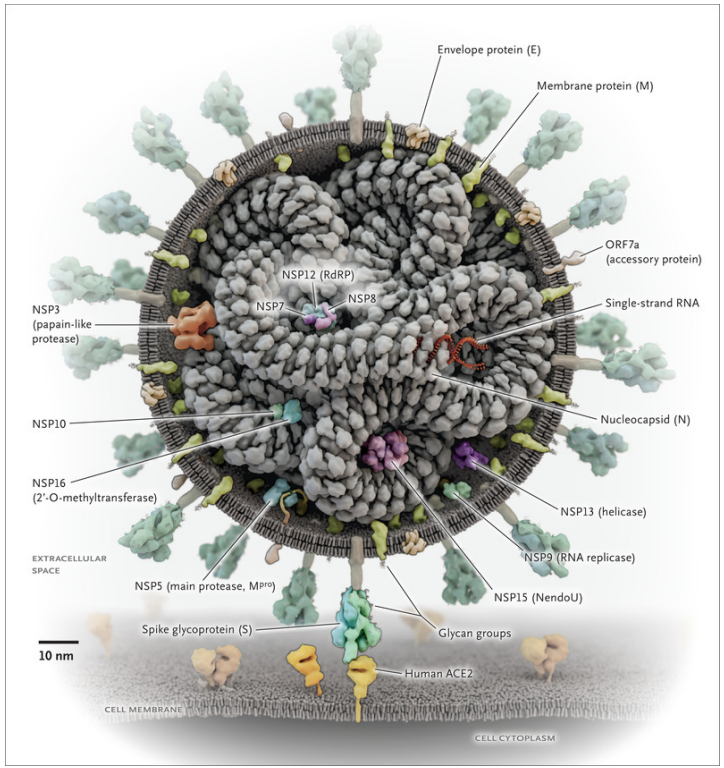

- Spike Protein (S)
  - Recognizes human angiotensin-converting enzyme 2 in the initial stage of infection
- Nucleocapsid Protein (NCAP or N)
  - Involved in (viral) replication and transcription of the genome
  - Structural component of the viral particle
  - Essential for survival

Protein	Intensity	Percentage	MW (kDa)
P0DTC9|NCAP_SARS2	3.1998E+11	88.6	46
P0DTC2|SPIKE_SARS2	17745000000	4.9	141
P0DTC5|VME1_SARS2	13582000000	3.8	25
P0DTD1|R1AB_SARS2	4608000000	1.3	794
P0DTD2|ORF9B_SARS2	2910500000	0.8	11
P0DTC3|AP3A_SARS2	1941200000	0.5	31
P0DTC6|NS6_SARS2	307510000	0.1	7
P0DTC4|VEMP_SARS2	49604000	0.0	8

https://www.nejm.org/doi/full/10.1056/NEJMcibr2007042
https://www.biorxiv.org/content/10.1101/2020.04.23.057810v1.full.pdf

Why LC-MS for SARS-CoV-2 Analysis?

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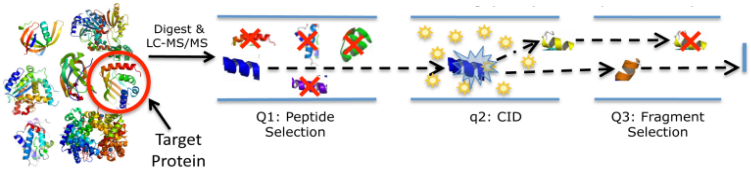
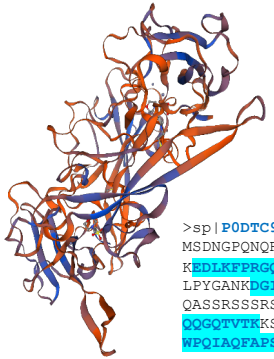
- SARS-CoV-2 is a protein-rich viral particle
  - Orthogonal quantitative read-out of digested peptides that correspond to the protein(s) of interest
  - MS based detection compatible with conventional small molecule quantitative systems

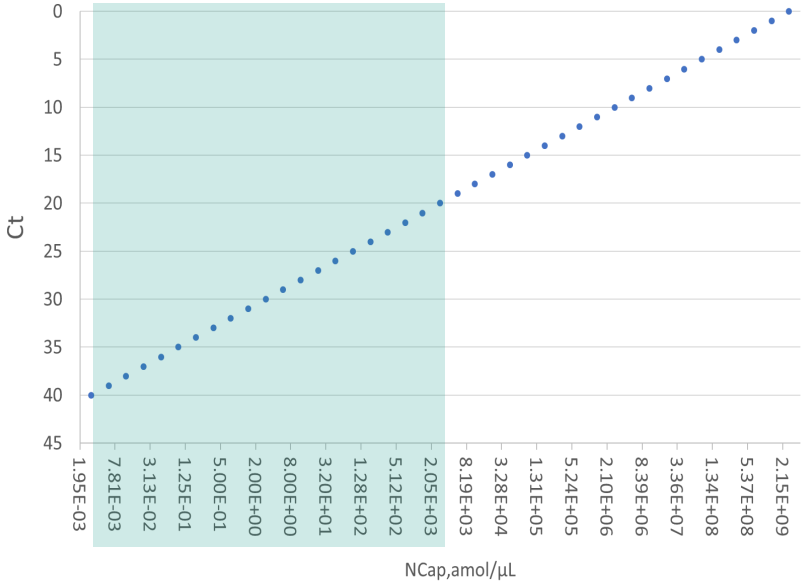


- Multi-analyte detection capabilities; other viruses or biomarkers could potentially be detected within the same experiment



>sp|P0DTC9|NCAP_SARS2 Nucleoprotein
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
LPYGANKDGIIWVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGS
QASSRSSSRSRNSSRNSTPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQ
QQGQTVTKKSAAEASKKPRQKRTATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKH
WPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAY
KTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADSTQA

Converting Cts to mols for LC-MS



Ct	*Viral Load, RNA/µL	*NCAP Protein amol/µL	*NCAP Peptide amol/µL
20	$1.74x10^6$	2,900	2,900
25	$5.59x10^4$	92.8	92.8
30	$1.79x10^3$	2.97	2.97
35	57.4	0.0953	0.0953
40	1.84	0.00305	0.00305

*estimates based on cell culture studies

SARS-CoV-2 Workflow Strategies

Enriched and Non-Enriched Workflows

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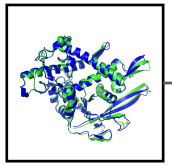
Approach | **Sample Collection** | **Sample Processing** | **Detection** | **Pros / Cons**

Non-Enriched



Extract & Digest
Denature and digest

Direct Analysis
Inject digest

Enriched

Enrichment
SPE to clean digest
Peptide Antibody Affinity

UPLC-MS/MS (MRM)

- Higher background
- Lower sensitivity
- Longer LC run time
+ Broad range of markers
+ Good immediate availability

+ Cleanest background
+ Highest sensitivity
+ Shorter LC run time
- Limited range of markers
- Limited immediate availability
- Polyclonal → Monoclonal

Waters SARS-CoV-2 LC-MS Workflow

Research Use Only

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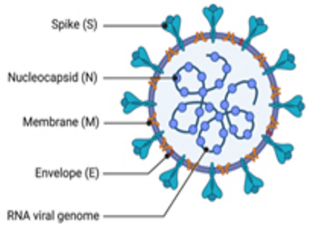
- **Direct detection and quantification** of 3 signature NCAP peptides: ADE, AYN, NPA
- **NCAP proteins**: most abundant and least prone to genetic mutation
- **No replication** of target analytes: reduced contamination and erroneous results

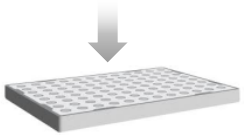


Sample Prep

Protein denaturation and digestion

Sample Enrichment

Antibody Magnetic Beads



Andrew
OneLab

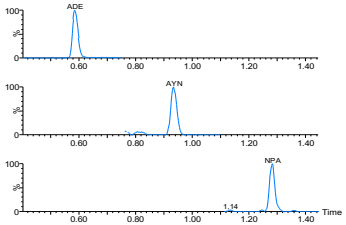
Enrichment with anti-peptide antibody magnetic beads and Andrew +

Detection

MAXPEAK™ PREMIER Columns

LC-MS/MS analysis
2.5mn run time

Results

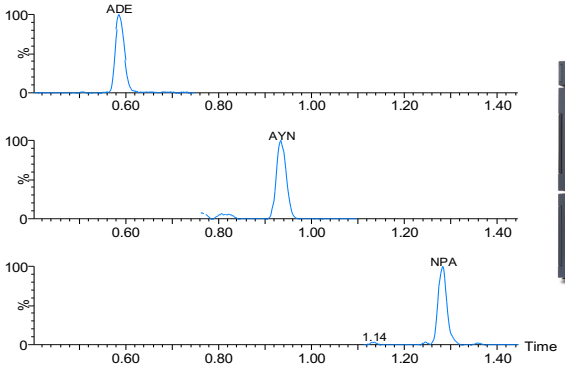


ADETQALPQR
AYNVTQAFGR
NPANNAAIVLQLPQGTTLPK

~4 hrs with automation → **~4 hrs LC-MS analysis**

LC-MS/MS Conditions

- ***ACQUITY UPLC I-Class FTN***
  - Column: ACQUITY Premier Peptide BEH C_{18}, 300Å, 1.7µm, 2.1mm x 30mm
  - Mobile Phase A: Water with 0.1% formic acid
  - Mobile Phase B: Acetonitrile with 0.1% formic acid
  - Column Temperature: 40°C
  - Injection volume: 20µL
  - Run time: 1.8 minutes (2.5 minutes cycle time)



- ***Xevo TQ-XS***
  - Ion mode: ESI+
  - Capillary Voltage: 0.5 kV
  - Desolvation Temperature: 600°C
  - Desolvation Gas: 1000L/Hr
  - Source Temperature: 150°C
  - Resolution: MS1 & MS2 0.75 FWHM

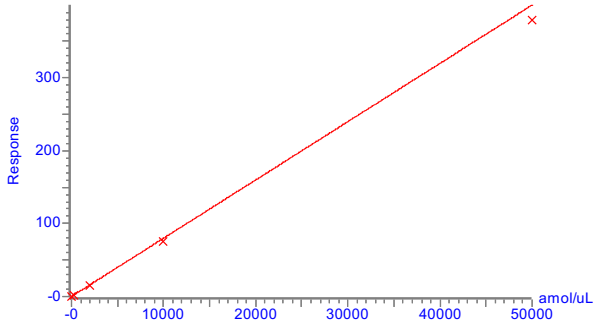
Peptide	MRM		Cone (V)	Collision (V)
ADE	564.8 > 400.2	Quantifier	35	19
	564.8 > 584.4	Qualifier	35	20
	569.8> 410.2	SIL	35	19
AYN	563.8 > 679.4	Quantifier	35	19
	563.8 > 892.5	Qualifier	35	19
	568.8 > 689.4	SIL	35	19
NPA	687.4 > 841.5	Quantifier	35	18
	687.4 > 865.5	Qualifier	35	23
	690.4 > 849.5	SIL	35	18
AYE*	588.8 > 400.2	Quantifier	35	19
	588.8 > 777.4	Qualifier	35	15
	569.8> 410.2	SIL (ADE)	35	19

*Delta variant mutation D377Y

Calibration Performance

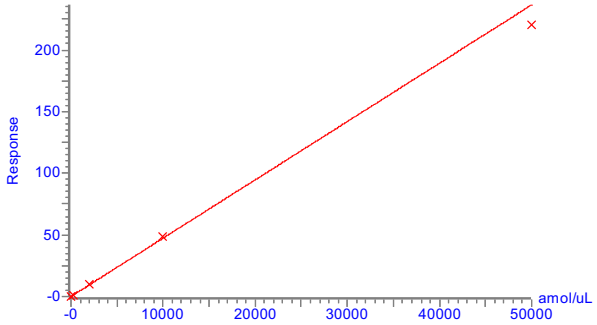
- Peptide calibration lines were prepared from 3 – 50,000 amol/µL in VTM (Virus Transport Media)
- Correlation coefficients (r^2) were >0.99

Correlation coefficient: r = 0.998833, r^2 = 0.997667
Calibration curve: 0.0079962 * x + 0.00127215
Response type: Internal Std (Ref 4), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: 1/x^2, Axis trans: None



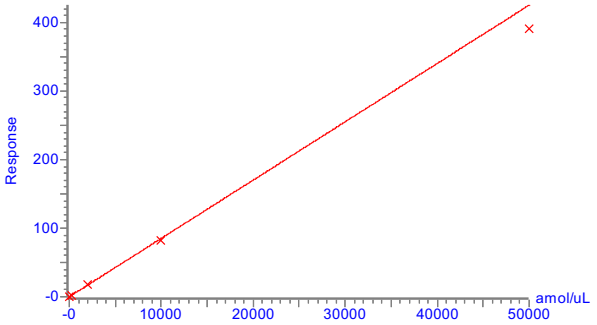
ADE

Correlation coefficient: r = 0.997037, r^2 = 0.994082
Calibration curve: 0.0047293 * x + 0.00113152
Response type: Internal Std (Ref 5), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: 1/x^2, Axis trans: None



AYN

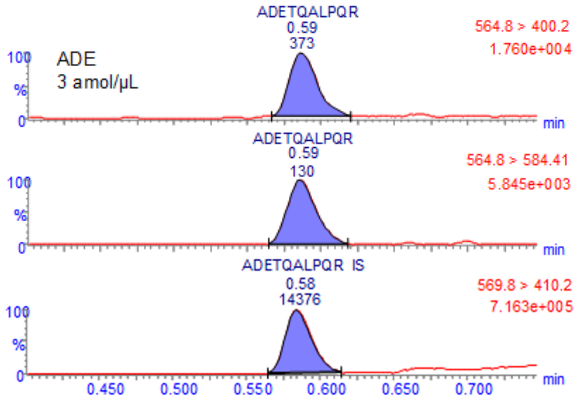
Correlation coefficient: r = 0.997277, r^2 = 0.994560
Calibration curve: 0.00850259 * x + -0.00154745
Response type: Internal Std (Ref 6), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Include, Weighting: 1/x^2, Axis trans: None



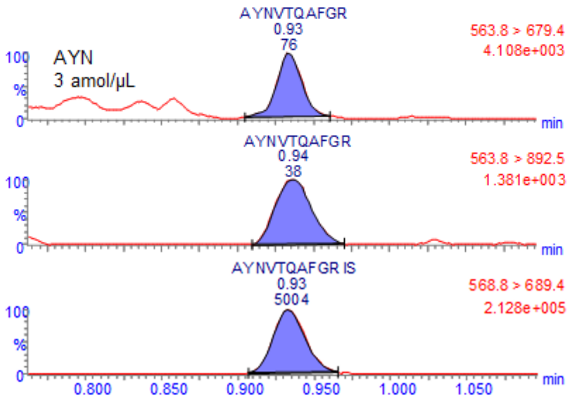
NPA

Analytical Sensitivity

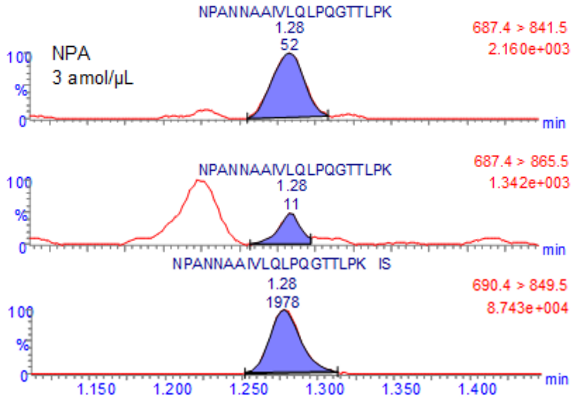
- Analytical sensitivity at 3 amol/µL* in VTM after sample enrichment for 3 peptides
  - ADE and AYN provide the greatest analytical sensitivity, with peaks detected in all qualifier traces
  - NPA provides lower analytical sensitivity, with much lower qualifier ion peak areas



ADE



AYN



NPA

*amol= 10^{-18} mole

Precision

NCAP Protein Derived Peptides and Spiked Peptides

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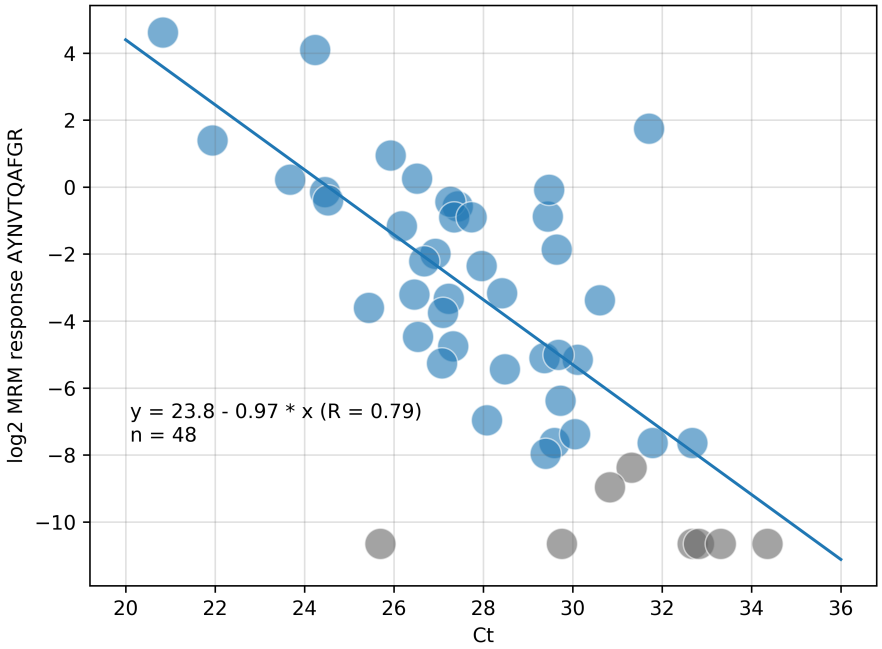
- In-house QCs using spiked NCAP and spiked peptides (ADE, AYN and NPA) into VTM at 3, 10, 400 and 25,000 amol/µL
  - Precision was performed over 5 separate occasions, with 5 replicates at each concentration
  - Spiked Peptides
    - Intra-day and inter-day precision was ≤17.4% CV at 3 amol/µL and ≤12.4% CV from 10 – 25,000 amol/µL
  - Spiked NCAP Protein
    - Intra-day precision was ≤18.8% CV at 3 amol/µL and ≤10.8% CV from 10 – 25,000 amol/µL
    - Inter-day precision was ≤18.8% CV from 3 – 25,000 amol/µL

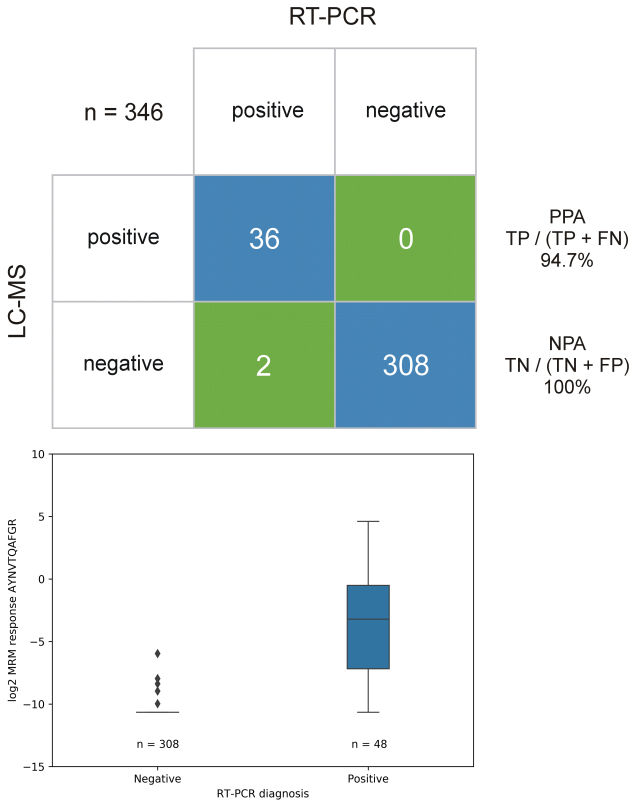
	Spiked Peptide (amol/µL)							
	Intra-Day Precision				Inter-Day Precision			
	3	10	400	25000	3	10	400	25000
ADE	9.0%	5.0%	2.0%	2.9%	12.9%	8.4%	2.6%	3.3%
AYN	12.5%	6.8%	2.4%	3.0%	15.2%	10.2%	6.8%	4.7%
NPA	17.1%	12.4%	3.0%	4.5%	17.4%	10.9%	5.2%	3.6%

	Spiked NCAP Protein (amol/µL)							
	Intra-Day Precision				Inter-Day Precision			
	3	10	400	25000	3	10	400	25000
ADE	18.4%	7.8%	2.5%	3.1%	11.5%	16.8%	18.8%	11.8%
AYN	13.2%	10.2%	2.4%	2.9%	11.6%	17.6%	18.5%	11.1%
NPA	18.8%	10.8%	4.1%	3.8%	11.6%	15.4%	18.2%	13.3%

Proof of concept

Results based on a similar workflow

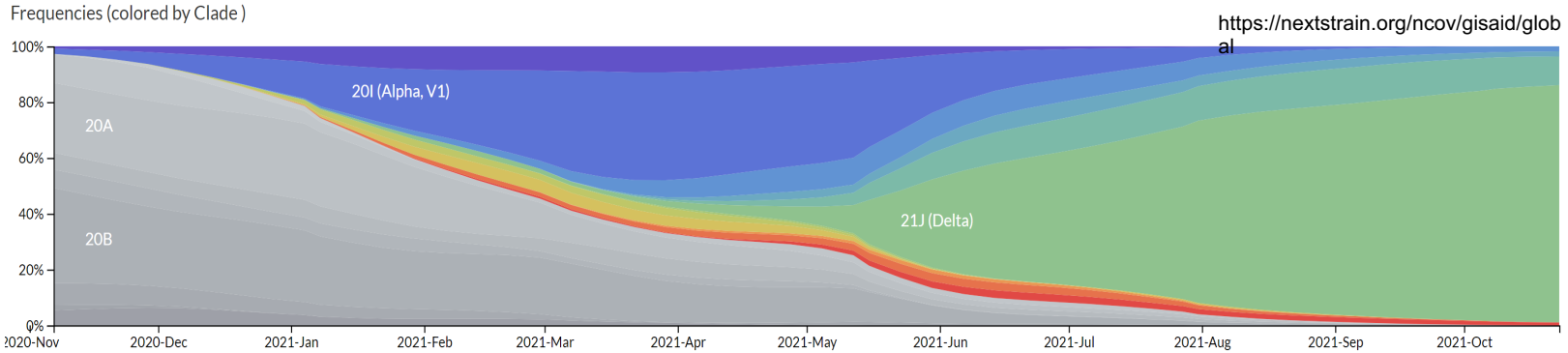




Rapid and Sensitive Detection of SARS-CoV-2 Infection Using Quantitative Peptide Enrichment LC-MS/MS Analysis. Hober et al. https://elifesciences.org/articles/70843

The Delta Variant

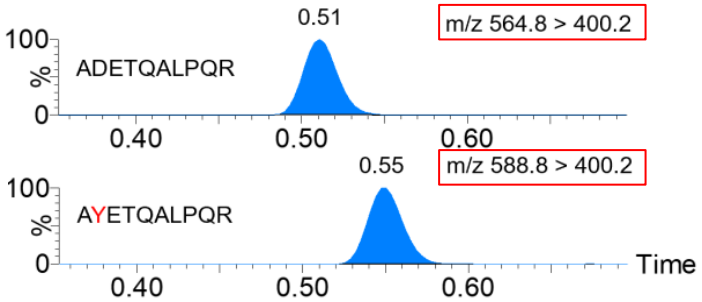
- As of July 2021, the SARS-CoV-2 Delta (B.1.617.2) variant rapidly became the dominant form of the virus across the globe

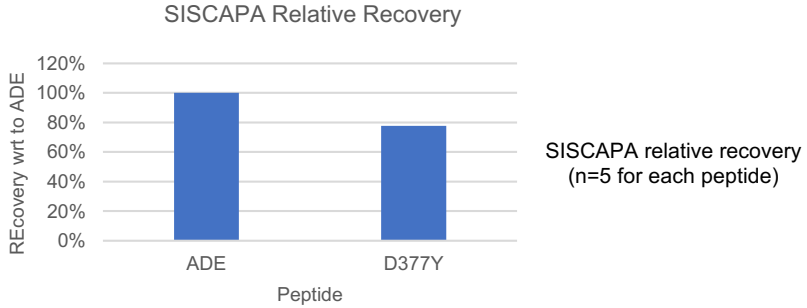


- Previous VoCs did not impact the selected peptides ADE, AYN and NPA
- Upon examining the Delta variant, it was noted that the ADE peptide was affected, with increased prevalence of an amino acid substitution adjacent to the N-terminus (D377Y)
  - Original ADE: ADETQALPQR
  - Variant D377Y: A**Y**ETQALPQR

Analysis of Delta Variant NCAP Peptides

- The variant peptide AYE (D377Y) was synthesized and added to a solution containing ADE, AYN and NPA
- All peptides were then added to viral transport medium (VTM) and extracted using the SARS-CoV-2 LC-MS Kit (RUO) and analyzed over a single experimental run
- Skyline was used to identify MRM transitions for the variant peptide and analysis was performed using the ACQUITY UPLC I-Class and Xevo TQ-XS





- No significant changes to the method are required to identify the new peptide target
- The variant peptide can be captured by the SISCAPA ADE wildtype monoclonal antibody
- SARS-CoV-2 LC-MS Kit (RUO) allows the **identification** of Delta variant and its **differentiation** from previous variants of concern in one experiment

Summary

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A kit-based LC-MS/MS method can be used to directly detect and quantify SARS-CoV-2 NCAP peptides for clinical research

The SARS-CoV-2 LC-MS Kit (RUO) allows direct detection and quantification of three SARS-CoV-2 NCAP peptides with excellent analytical sensitivity, analytical selectivity, reproducibility and speed.

LC-MS can be used as a complementary approach to PCR for research, with the added benefit of using the multi-analyte detection capabilities of LC-MS/MS to also monitor biomarkers as part of SARS-CoV-2 longitudinal research studies

Acknowledgements

- Waters – Chemistry, SciOps, UK Sales
- Cov-MS Consortium
- University Collaborations
- UK NHS
- SISCAPA