Unlocking Precision: InoKey™, A Disease-Agnostic Custom Discovery to Targeted Proteomics Solution for Drug Development

Introduction

Despite the profound impact of genomics and transcriptomics in revolutionizing healthcare and research, these technologies might not always provide robust predictions on which individuals will respond effectively to a given therapy. Proteomics, the ultimate assessor of cell and tissue health, offers a crystal clear picture of a patient's phenotype and provides substantial predictive potential. One critical gap remains: how do we bridge unbiased discovery proteomics findings and targeted proteomics assays to monitor dysregulated protein biomarkers with high precision and accuracy in the form of a clinically validated assay?

InoKey™ has been developed to offer an elegant and comprehensive solution to the challenge of identifying novel protein biomarkers and translating those findings into a functional assay with clinical utility. InoKey™ is an end-to-end discovery to targeted proteomics solution, supporting researchers every step of the way, from experimental design and planning to data acquisition, interpretation, reporting, and clinical utility **(Figure 1)**. InoKey™ currently supports preclinical and clinical researchers globally, streamlining their biomarker strategy for monitoring drug mechanism-of-action, efficacy, and patient stratification.

InoKey™ incorporates customized data-processing workflows, specialized sample preparation techniques, and novel internal standards to provide excellent reproducibility, robustness, sensitivity, and a linear dynamic range greater than six orders of magnitude, allowing for absolute quantification to the ng/mL level. A separate IP-MS solution is also available for absolute quantification of even lower-level markers down to the pg/mL range in a multiplex format.

The present case study demonstrates how InoKey™ formulated a high-throughput LC-MS/MS workflow to stratify patients' plasma proteomes in response to SARS-CoV-2 infection **(Figure 2)**. The resulting targeted MRM COVID-19 biomarker assay outperformed other severity score models and robustly predicted patient outcomes for a streamlined clinical treatment escalation strategy.

Definitions

Discovery Proteomics: Global, broad-scale proteomics analyses to identify and quantify novel biomarkers, therapeutic targets, mechanisms, and molecular pathways

Targeted Proteomics: Quantitative and selective tracking of predefined protein targets within a complex biological sample

Liquid Chromatography-Mass Spectrometry (LC–MS): Analytical technique that involves the separation of target compounds or analytes followed by their mass-based detection and quantification

Immunoprecipitation Mass Spectrometry (IP-MS): Analytical technique that involves enriching target biomarkers using immunoaffinity reagents, followed by LC-MS detection

Multiple Reaction Monitoring (MRM): Targeted mass spectrometry technique enabling the precise and simultaneous quantification of multiple proteins, peptides, metabolites, and small molecules

Experimental Design

1

Create your custom assay with our proteomics experts

Ship your samples to our CLIA, FDA-inspected (US) or GCP/CLP (UK)-certified labs

Bioinformatics Analysis

2

Our bioinformatics team analyzes data through proprietary analytical algorithms and workflows, utilizing internal standards

In accordance with FDA guidelines for bioanalytical method development

Data Interpretation

3

Upon completion, our expert team provides detailed reporting and analysis of results to guide next steps and decision-making

Method Overview

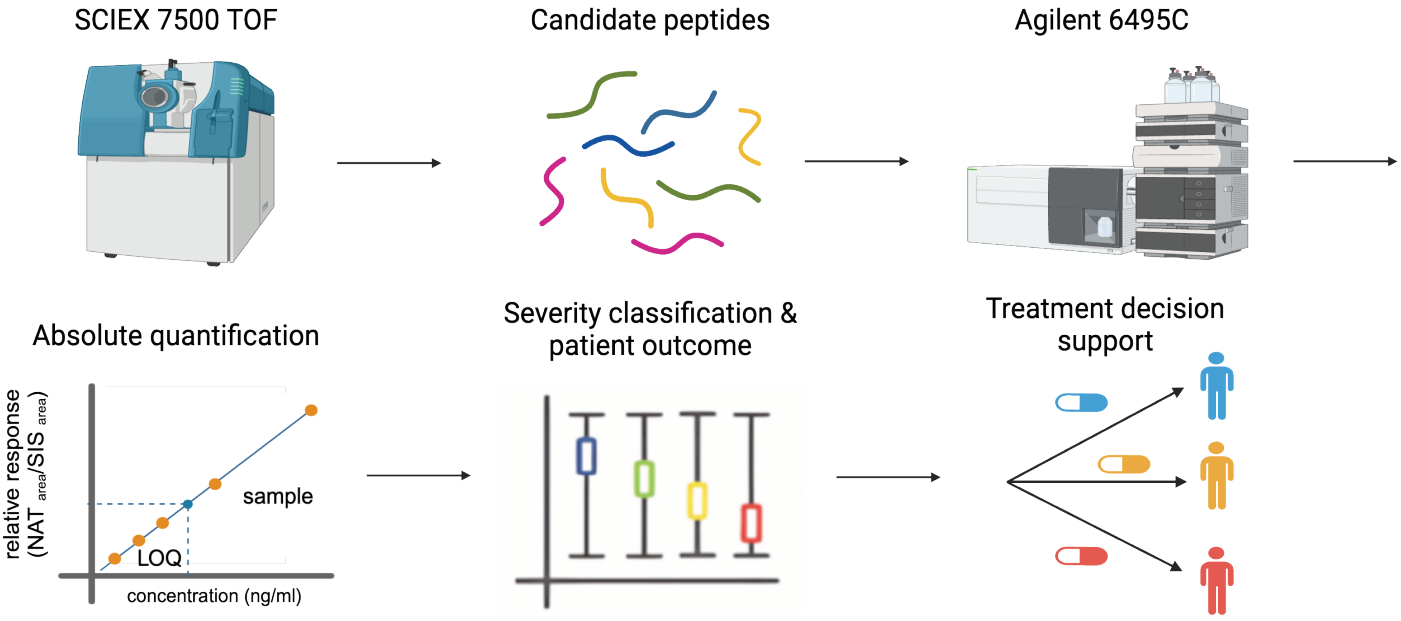


Figure 2. Schematic overview of the LC-MS/MS workflow, from discovery proteomics, peptide selection to the development and application of a targeted MRM COVID-19 biomarker assay in a clinical setting. Top panel: Discovery proteomics in a deeply phenotyped COVID-19 patient population revealed strong candidate peptides/biomarkers based on their discriminating power and robust analytical performance. Bottom panel: A targeted LC-MS MRM assay was developed for absolute biomarker quantification using synthetic calibrators and isotope-labeled internal standards. The assay was applied to plasma samples from different COVID-19 patient cohorts to determine severity classification and clinical outcome, allowing streamlined treatment decision workflows and future clinical trial optimization.

Results

Discovery proteomics revealed distinct clusters of clinically relevant biomarkers in various disease states

50 peptides corresponding to 30 plasma proteins were identified and selected through a discovery proteomics approach in plasma samples acquired from a deeply phenotyped COVID-19 patient cohort (n=139) **(Figure 3)**. Biomarkers were selected based on different prognostic factors, including their association with the host response, such as inflammatory and innate immune response, complement cascade, and coagulation[1,2].

Succesful development and characterization of a robust MRM-based targeted COVID-19 biomarker assay

Peptides determined from discovery proteomics studies informed the development of a MRM-based targeted COVID-19 biomarker test for use as a clinical assay[3]. To investigate the assay's performance and utility, the selected peptides' performance was investigated, including intra- and inter-batch repeatability, quantification limit, linearity, and accuracy **(See Table)**.

Representative Peptides & Performance:

	Low Concentration			High Concentration		
Peptide	Inter-Batch CV	Intra-Batch CV	Accuracy	Inter-Batch CV	Intra-Batch CV	Accuracy
1	6.8	4.2	94.4	4.5	0.9	98.2
2	6.8	4.6	93.6	4.4	1.7	98.4
3	2.7	2.0	99.4	4.5	0.3	99.7
4	10.7	8.5	87.6	5.0	1.6	99.3
5	12.5	10.1	96.9	6.5	2.0	97.8

**values reported in % unless otherwise stated*

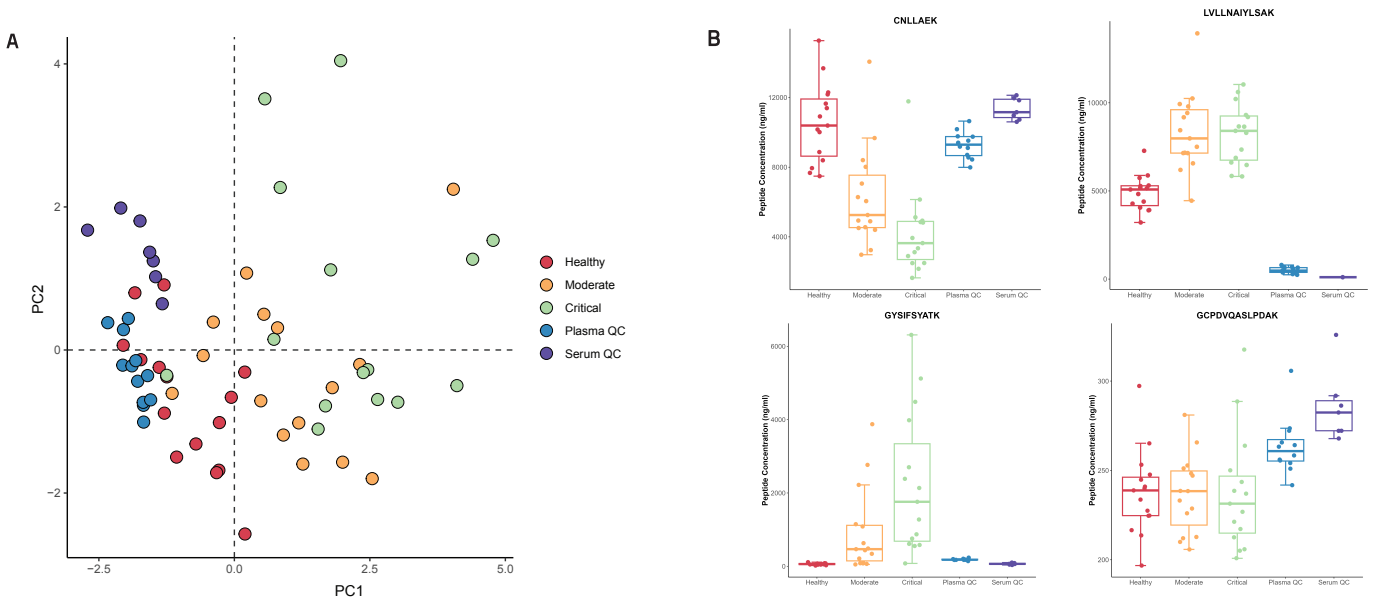


Figure 3. (A) A reproduction of Figure 3 from Lane *et al.* PCA biplot quantification results demonstrating COVID-19 disease stratification and patient clustering. **(B)** A reproduction of Figure 4 from Lane *et al.* Example box plots of surrogate peptides with levels significantly changing across COVID-19 disease states.

The COVID-19 MRM assay succesfully classified disease severity and outcome prognosis in a COVID-19 cohort

Next, the chosen peptides were tested for their utility in classifying COVID-19 treatment escalation level, a proxy for disease severity[4]. The markers displayed robust changes in abundance between the different patient WHO groups **(Figure 4)** and indeed classified patients into different groups **(Figure 5A)**. The assay's prognostic value was then established in a separate larger, longitudinal cohort. Based on the biomarker data, patients' WHO grade predicitions were in agreement with the actual treatment escalation. The targeted COVID-19 assay also outperformed other severity score models, strongly predicted COVID-19 patients' outcomes, and classified them according to their WHO grades **(Figure 5B and 5C)**.

Conclusion

- Unbiased discovery proteomics revealed candidate COVID-19 disease severity biomarkers based on distinct patient clustering profiles
- The COVID-19 biomarkers demonstrated excellent analytical performance and therefore advanced for further development
- Using LC-MS/MS, an MRM-based targeted COVID-19 biomarker assay was analytically validated and developed for use in a clinical cohort
- The assay outperformed severity score models, strongly predicted patient outcomes, and classified them according to WHO grades

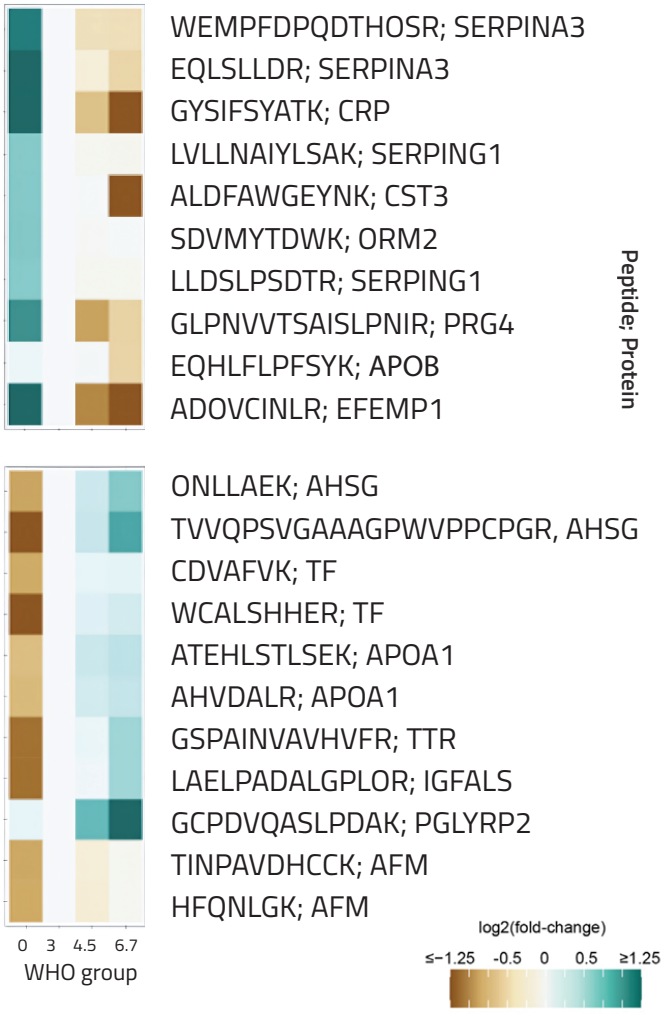


Figure 4. A reproduction of Figure 4A from Wang *et al.* The targeted COVID-19 biomarker assay reproducibly and reliably reports COVID-19 disease severity in a patient cohort. Heat map displaying the log2 fold-change of the peptide/biomarker vs its median concentration in patients classified according to their severity score by WHO group.

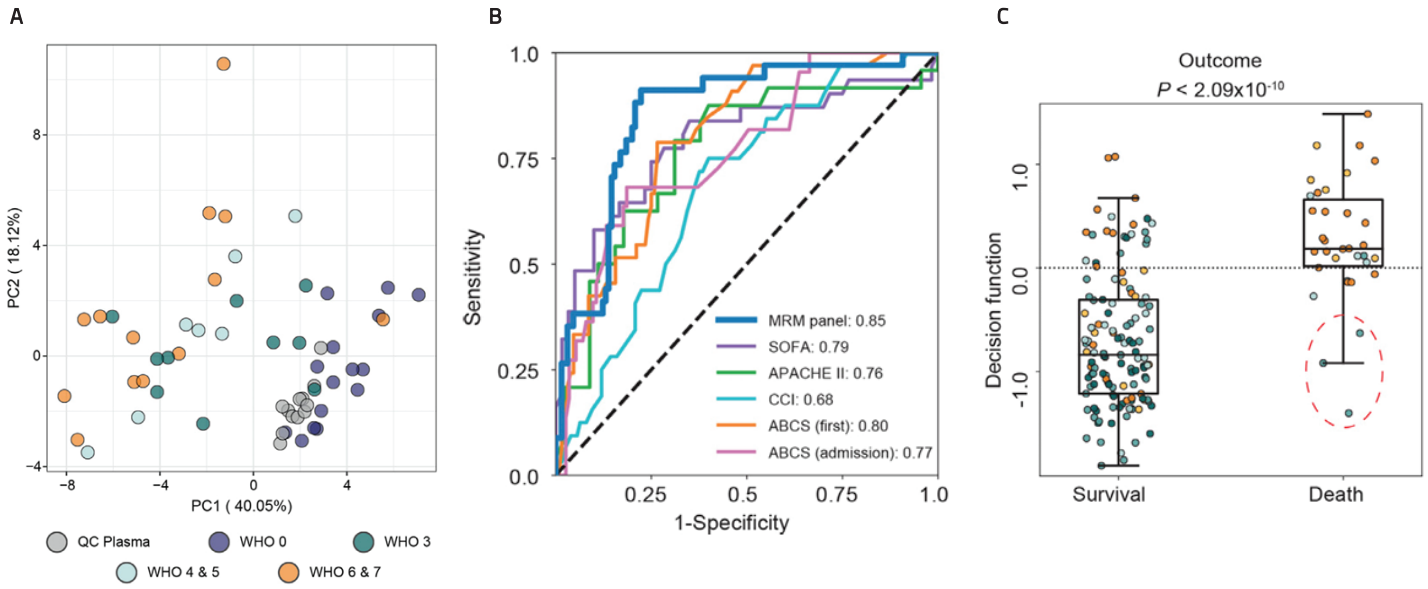


Figure 5. (A) A reproduction of Figure 3C from Wang *et al.* PCA biplot based on the absolute quantification of chosen biomarkers, demonstrating distinct separation clusters between the different patients according to their WHO classification. **(B)** A reproduction of Figure 5A from Wang *et al.* Receiver operating characteristic (ROC) curve for the prediction of survival against non-survival, from a single plasma sample. The blue curve denotes targeted MRM COVID-19 biomarker assay against other severity score models. **(C)** A reproduction of Figure 5B from Wang *et al.* Decision function displayed as boxplots for every patient, categorised according to their outcome and colored individually with respect to their WHO grade at the day the sample was taken. The red circle denotes patients who died but were predicted with high survival rate. These patients had do-not-intubate orders due to other pre-existing medical conditions, and therefore were not escalated for ventilation therapy. WHO group 1: healthy; WHO group 3: COVID-19 individuals requiring hospitalisation but no oxygen therapy; WHO group 4: COVID-19 individuals requiring hospitalisation and non-invasive oxygen therapy; WHO group 6: Severely affected COVID-19 individuals requiring mechanical ventilation.

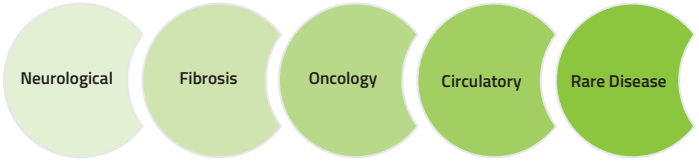
References

1. High throughput LC-MS/MS stratification of plasma proteome response to SARS-CoV-2 infection. Lane, Catherine et al. Sciex technical note
2. Demichev V, Tober-Lau P, Lemke O, et al. A time-resolved proteomic and prognostic map of COVID-19. Cell Syst. 2021
3. A multiplex protein panel assay for severity prediction and outcome prognosis in patients with COVID-19: An observational multi-cohort study. Wang, Ziyue et al. *eClinicalMedicine*, Volume 49, 101495
4. World Health Organization. R&D Blueprint Novel Coronavirus COVID-19 Therapeutic Trial Synopsis (WHO). 2020.

Connect with Us

Our disease-agnostic InoKey™ platform technology is now commercially available and supporting researchers globally to monitor drug mechanism-of-action, efficacy and patient stratification.

Bridge your translational biomarker gap with InoKey™. Select your panel and ship your sample, we'll handle the rest.



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