Developing a Quick and Robust Mass Spectrometry-based Method for the Detection of SARS-CoV-2

Richard J. Gibson[1], Stephanie N. Samra[1], Kerry M. Hassell[1], George A. Renney[2], Sarvesh Iyer[1], Yang Pengxiang[1], Luan Shen[1], Bradley J. Hart[1]

1. **Thermo Fisher Scientific, San Jose, California.**
2. **Thermo Fisher Scientific, Hemel Hempstead, United Kingdom.**

ABSTRACT

Purpose: Developing a bottom-up proteolytic workflow could allow for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

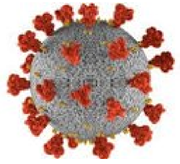
Methods: This quick and robust mass spectrometry-based method for the detection of such biomarkers targets six peptides from recombinant protein samples that were spiked onto nasopharyngeal swabs, or into saliva, placed in viral transport media and enzymatically digested.

Results: Sub/low-femtomole on column detection and quantification limits were observed for each peptide.

INTRODUCTION

SARS-CoV-2 is a highly infectious virus that has resulted in over 4 million deaths.[1] Containing the virus has only had limited success, partly due to its spread by asymptomatic carriers, emphasizing the need for widespread testing.

Polymerase chain reaction (PCR) has proven to be the gold standard method in the detection of COVID. Although PCR has demonstrated high sensitivity (80 %) and specificity (> 98 %),[2] a shortage of reagents and trained scientists resulted in a backlog of tests and inconsistent processing times during the height of the pandemic. This highlights the need to develop orthogonal methods to create a robust and economical system capable of sufficient testing for future infectious disease outbreaks.



P0DTC2 – Spike

P0DTC4 – Envelope

P0DTC6 – Membrane

P0DTC9 – Nucleocapsid

Figure 1. The SARS-CoV-2 viral particle contains numerous copies of proteins

Bottom-up mass spectrometry (MS) could confirm positive COVID cases by detecting peptides from COVID proteins (Figure 1). Enzymatic digestion of proteins produces peptides that can be separated by liquid chromatography (LC). These peptides are easier to identify than intact proteins (due to their size) and numerous peptides can be targeted. Consequently, a mass spectrometry-based COVID peptide quantification method was created to complement PCR methods.

MATERIALS AND METHODS

Sample Preparation: An absolute peptide quantification method was developed using a Thermo Scientific™ Vanquish™ MD HPLC system and a TSQ Altis™ MD mass spectrometer (Figure 2). Recombinant SARS-CoV-2 proteins were spiked into pooled nasal fluids and saliva, before being added to viral transport media. Samples were then precipitated, centrifuged and enzymatically digested (Thermo Scientific™ SMART Digest™ Trypsin kit).

LC/MS Methods: Resulting peptides were separated by a 4-minute LC run with a Hypersil GOLD™ C18 column (1.9 µm, 2.1 x 50 mm), coupled with a single reaction monitoring method.

Data Analysis: Data was analyzed by Thermo Scientific™ TraceFinder™ LDT software.

Figure 2. Thermo Scientific Vanquish MD HPLC and TSQ Altis MD mass spectrometer

RESULTS

Table 1. Optimized SRM transitions, collision energies, LODs and LOQs for peptides from SARS-CoV-2 protein digests

Peptide Sequence	Peptide Mass (Da)	Retention Time (minutes)	Unlabeled Peptide			Nasal Samples		Saliva Samples	
			Q1 (Da)	Q3 (Da)	CE (eV)	LOD (fmol)	LOQ (fmol)	LOD (fmol)	LOQ (fmol)
KADETQALPQR	1256.659	1.52	419.558	400.23 673.32 744.35	12	0.25	0.5	0.25	1.0
ADETQALPQR	1128.564	1.59	564.786	400.23 513.31 584.35	20	0.25	0.5	0.25	0.5
AYNVTQAFGR	1126.564	1.95	563.786	679.35 778.42 892.46	20	0.25	0.5	0.25	0.5
NPANNAAIVL QLPQGTTLPK	2060.150	2.22	1030.579	841.48 1082.62 1195.71	33	2.5	5.0	2.5	2.5
GWIFGTTLDSK	1224.626	2.30	612.817	868.44 664.35 721.37	22	5.0	10.0	5.0	10.0
DGIIWVATEGALNTPK	1684.890	2.32	842.949	1001.53 1100.60 1286.67	24	2.5	2.5	2.5	5.0

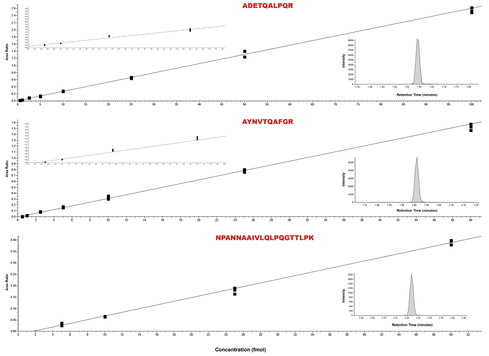


Figure 3. Spectra and calibration curves for targeted peptide from nasal fluid samples

Absolute quantitation of six targeted peptides (Table 1) allowed LODs/LOQs to be determined (Figure 3). Five of the chosen peptides were from the nucleocapsid and one was from the spike protein, allowing the detection of biomarkers from two different proteins.

Clear chromatographic separation was observed for each of the five nucleocapsid peptides (Figure 4). All peptides displayed minimal variance in retention time and an almost identical retention time (± 0.01 minutes) to the corresponding stable isotope-labeled standards.

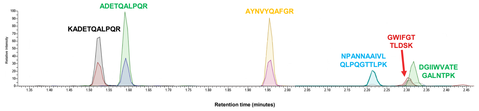


Figure 4. A chromatographic trace demonstrating the separation of the targeted peptides

CONCLUSIONS

A robust, quick and reliable absolute peptide quantitation assay has been developed to detect SARS-CoV-2 using a Vanquish MD HPLC and a Altis MD mass spectrometer. Optimal conditions were determined for the five best performing nucleocapsid peptides and the best performing spike peptide. Detection limits were determined to be between 0.25 and 5.0 fmol on column, with quantitation limits of between 0.5 and 10.0 fmol.

- **Limits of detection as low as 0.25 fmol on column**
- **Limits of quantitation as low as 0.5 fmol on column**
- **Reliable quantitation of six peptides from SARS-CoV-2**
- **Collection of data using medical devices**

REFERENCES

1. Coronavirus in the US: Latest Map and Case Count: www.nytimes.com/interactive/2020/us/coronavirus-us-cases.html.

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TRADEMARKS/LICENSING

Richard.Gibson@thermofisher.com

PO66092 EN0921S