Rapid and Generic Identification of Respiratory Viruses with Mass Spectrometry

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Summary

Tested respiratory viruses: influenza A virus, respiratory syncytial virus (RSV) and human metapneumovirus (hMPV), were successfully identified directly from crude cell cultures using liquid chromatography-tandem MS (LC-MS/MS).

The identification was preceded with the simple, generic and fast sample preparation method. The determined identification limit was only one order of magnitude higher than required for identification from a clinical specimen. Because identification is based on amino acid sequences deposited in GenBank, other viruses are likely to be identified using the developed method. Further improvement of the sample preparation method, may soon bridge the gap between research and clinical diagnostics.

We conclude that MS-based techniques have the potential to become a complementary method to PCR in viral diagnostics.

Introduction

Rapid detection and identification of threatening microorganisms is essential for the effective response to an infectious disease outbreak. LC-MS/MS is a generic method that can rapidly detect and identify microorganisms. Thus far, this technique has not been used for viral diagnostics. Respiratory viruses are a major cause of infections. Especially influenza is an important cause of morbidity and mortality in humans during seasonal outbreaks, epidemics and pandemics. Other examined viruses in this study, RSV and hMPV, are a cause of common colds. The aim of this study was to determine whether LC-MS/MS has an applicable potential for diagnostics of respiratory viruses. Therefore, the identification of influenza A and other respiratory viruses directly from titrated cultures was performed subsequently after a generic, simple and fast sample preparation procedure.

Results

All analysed viruses, namely influenza A (**Table 1**), hMPV and RSV (data not shown), were identified with LC-MS/MS.

Table 1. Sequence coverage of the identified proteins

Influenza A proteins[a]	Virus titer (total genome copies)			
	$7x10^9$	$7x10^8$	$7x10^7$	$7x10^6$
Nucleoprotein (NP)	~79	~54	~43	~17
Matrix protein (M1)	~77	~50	~34	0
Non-structural protein (NS1)	~65	~41	~21	0
Hemagglutinin (HA)	~17	~16	0	0
Neuraminidase (NA)	0-22	0-12	0	0

[a] A protein was considered as identified, when the Mascot score for the protein was ≥ 50 and a minimum of three peptides, of score ≥ 20, were identified for that protein.

Amino acid sequence variation in NP is enough to discriminate between clinical most relevant influenza subtypes. In **Figure 1** the sequence diversity between Influenza A H1N1 and H3N2 is shown. Marked in red are the detected NP peptides at 7 x 10^6 genome copies in a total volume of a sample injected into LC-MS/MS.

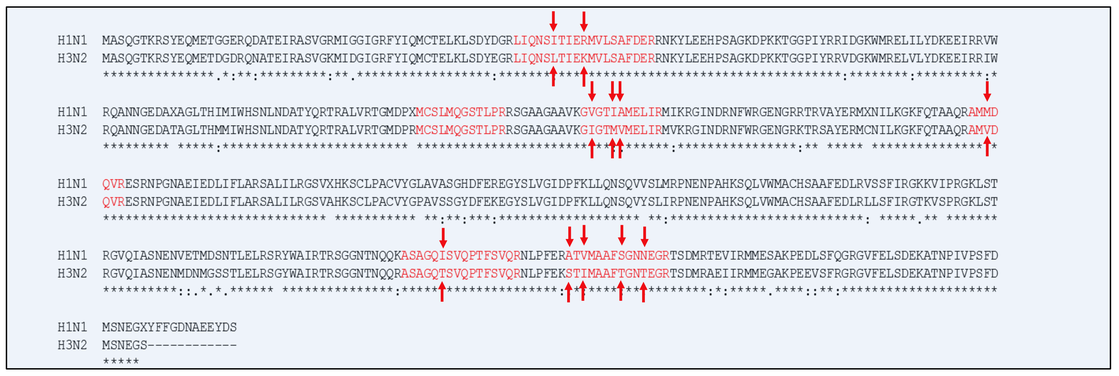

Figure 1. Nucleoprotein sequence alignment of influenza A H1N1 and H3N2.
In red, peptides detected with LC-MS/MS at 7 x 10^6 genome copies . Red arrows, detected a.a. differences between influenza A H1N1 and H3N2.

Mixtures containing two different viruses: mixture A - influenza H3N2 and RSV, and mixture B - influenza H1N1 and hMPV, were analyzed with LC-MS/MS. All the tested viruses were simultaneously identified **(Table 2)**.

Table 2. Identified proteins of three different respiratory viruses from their serial dilutions.

Mix	Titer[a]	Id. virus[b]	Average sequence coverage of identified proteins[c] (%)							
			NP	M1	NS1	HA	NA	M2-1	F	P
A	10^9	Influenza A/H3N2	63	74	16	15	4	-	-	-
		RSV	21	43	-	-	-	53	9	55
	10^8	Influenza A/H3N2	48	49	20	13	7	-	-	-
		RSV	23	40	-	-	-	46	3	53
	10^7	Influenza A/H3N2	18	34	0	5	0	-	-	-
		RSV	14	0	-	-	-	23	0	0
B	10^9	Influenza A/H1N1	73	67	60	0	0	-	-	-
		hMPV*	14	29	-	-	-	10	15	16
	10^8	Influenza A/H1N1	49	33	39	0	0	-	-	-
		hMPV*	8	18	-	-	-	10	9	18
	10^7	Influenza A/H1N1	24	10	11	0	0	-	-	-
		hMPV*	0	0	-	-	-	0	0	0

[a] Total genome copies number of the tested influenza virus and RSV. *The starting concentration of hMPV was 100-fold lower than that of influenza and RSV. [b] Identified virus. [c] An average (calculated from two independent experiments) sequence coverage of identified by Mascot proteins. A protein was considered as identified when the Mascot score was ≥ 50 and a minimum of three peptides, of score ≥ 20, were identified in the protein.

NP, nucleoprotein; M1, matrix protein; NS1, non-structural protein 1, only in influenza; HA, hemagglutinin, and NA, neuraminidase, only in influenza; M2-1, matrix protein, F, fusion glycoprotein, and P, phosphoprotein, only in RSV and hMPV.

Materials and Methods

Cultures of ten human influenza A (8 H1N1 and 2 H3N2) viruses, four hMPV and four RSV strains, were tested. Additionally, two 1:1 mixed virus samples, mixA: H3N2 and hMPV and mixB: H1N1 and RSV, were analysed.

Schematic presentation of the used sample preparation method:

Viral culture → +RapiGest → Incubation at 97°C → +DTT → +ACN → Incubation at 60°C → Centrifugation → +Trypsin → Enzymatic reaction in REDS → +FA → Incubation at 37°C → Centrifugation → LC-MS/MS

DTT, dithiothreitol; ACN, acetonitrile; REDS, a Rapid Enzymatic Digestion System; FA, formic acid; LC-MS/MS, a nano-Advance LC system (Bruker) coupled to a Q-TOF mass spectrometer (maXis impact, Bruker).

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