Journal of Virological Methods 152 (2008) 117–121

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

Simultaneously subtyping of all influenza A viruses using DNA microarrays

Han Xueqing[a,*,1], Lin Xiangmei[a,1], Liu Bohua[b], Hou Yihong[a], Huang Jinhai[c], Wu Shaoqiang[a], Liu Jian[a], Mei Lin[a], Jia Guangle[a], Zhu Qingyu[b,*]

[a] Chinese Academy of Inspection and Quarantine, Beijing 100029, China
[b] State Key Laboratory of Pathogens and Biosecurity, Institute of Microbiology and Epidemiology, Beijing 100071, China
[c] Tianjin University, Tianjin 300072, China

ARTICLE INFO

Article history:
Received 17 November 2007
Received in revised form 12 May 2008
Accepted 15 May 2008
Available online 18 July 2008

Keywords:
Influenza A virus
Subtyping
Microarray

ABSTRACT

Rapid diagnosis of novel emerging subtypes of influenza viruses is vital for effective global influenza surveillance. To this end, a novel microarray based surveillance was developed for subtyping all influenza A viruses on one chip. Using reference strains of different influenza subtypes and samples from different areas, the results show that all the subtypes of the influenza A virus could be identified simultaneously on this microchip with high sensitivity. There was no cross-hybridization reaction with other viruses, indicating that the microarray is specific for influenza A viruses. Such a diagnostic microarray will undoubtedly be useful for identifying novel influenza A virus subtypes.

Influenza A viruses are important human and animal respiratory pathogens which are responsible for both seasonal influenza outbreaks and periodic world-wide pandemics (Wright and Webster, 2001). Influenza A viruses have numerous subtypes; so far, sixteen serotypes of hemagglutinin (HA) (H1–16) and nine (N1–9) of neuraminidase (NA) have been identified in mammalian and avian species. Influenza A viruses have the characteristic of extensive genetic variation. They have a segmented genome consisting of eight separate RNA molecules. Upon co-infection in a cell, the viruses can exchange segments, leading to a diversity of reassortant strains, which has resulted in the continuous emergence of new virus variants which cause seasonal outbreaks of influenza. These present an important diagnostic problem; therefore, rapid diagnosis of novel emerging influenza viruses is essential for effective global influenza surveillance (McCullers et al., 1999; Debouck and Goodfellow, 1999). Reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR are currently available for differentiating specific subtypes of influenza A viruses. However, a major limitation of these PCR-based methods is that they do not provide information about other subtypes.

Microarrays have emerged as powerful tools for studying complex biological systems, since they enable theoretically simultaneous screening of tens of thousands of nucleic acid sequences. The microarray technology shows great promise for a wide range of possible applications, including drug discovery (Debouck and Goodfellow, 1999), detection of mutations (Schena et al., 1996), and genome mapping (Schena et al., 1996; Lashkari et al., 1997; Ivshina et al., 2004). Several studies have explored the use of diagnostic microarrays for influenza detection and subtyping (Kessler et al., 2004; Lodes et al., 2006; Townsend et al., 2006; Sengupta et al., 2003); however, the maximum number of influenza subtypes detectable in a single microchip is still relatively small. The development of a microarray-based strategy is described for identification of influenza virus subtype and the potential discovery of novel subtypes.

Influenza A virus strains of all 25 subtypes were obtained from the Zhuhai Entry–Exit Inspection and Quarantine Bureau, State Key Laboratory of Pathogens and Biosecurity, Institute of Microbiology and Epidemiology, and the Animal Influenza Laboratory of the Ministry of Agriculture. Viral RNAs were extracted by using the QIAamp Viral RNA Mini Kit (produced by QIAGEN, Valencia, CA, USA) and were stored at −70 °C.

Based on the genomic nucleotide sequences of influenza A viruses published in the GenBank database, 25 pairs of primers specific for different subtypes and one pair of general primers were designed using DNAstar, Bioedit, Primer 5.0, and OMIGA software

* Corresponding authors. Tel.: +86 10 64912741; fax: +86 10 64927408.
E-mail address: hanxueq@yahoo.com.cn (X. Han).
[1] These authors contributed equally to this work.

0166-0934/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2008.05.027

Table 1
Specific primers of AIV for gene microarray detection

Subtype	Primer no.	Primer sequence (5′–3′)	GenBank accession no. of reference gene	Product size
General primer	PMA-06001-uf	TCACTTGCTTCCGTTGAGG		
	PMA-06002-ur	TAMRA-GGTTTCGGATGTTACAGCGT		
H1	PMA-06003-H1f	TCACTTGCTTCCGTTGAGGGGAGCAATTGAGTTCAGTATC	CY002688	601 bp
	PMA-06004-H1r	TAMRA-GGTTTCGGATGTTACAGCGTGACACTCTCCTATTGTGACTG		
H3	PMA-06005-H3f	TCACTTGCTTCCGTTGAGGTGTTACCCTTATGATGTGCC	CY003712	669 bp
	PMA-06006-H3r	TAMRA-GGTTTCGGATGTTACAGCGTCCCTGTTGCCAATTTCAGAG		
H5	PMA-06007-H5f	TCACTTGCTTCCGTTGAGGAGTGAATTGGAATATGGTAACTG	AY770079	380 bp
	PMA-06008-H5r	TAMRA-GGTTTCGGATGTTACAGCGTAACTGAGTGTTCATTTTGTCAAT		
H6	PMA-06017-H6F	TCACTTGCTTCCGTTGAGGAAGGCACTTATTGGRTCAGG	DQ376650	685 bp
	PMA-06018-H6R	TAMRA-GGTTTCGGATGTTACAGCGTGTCCTCTAGTTTCAATCTGTGG		
H7	PMA-06009-H7f	TCACTTGCTTCCGTTGAGGTCAGGWTCTTCWTTCTATGC	AY831668	641 bp
	PMA-06010-H7r	TAMRA-GGTTTCGGATGTTACAGCGTTCYCCTTGTGCATTTTGATG		
H9	PMA-06011-H9f	TCACTTGCTTCCGTTGAGGAAGAGAATGGTCCTACATCGT	AY664671	493 bp
	PMA-06012-H9r	TAMRA-GGTTTCGGATGTTACAGCGTGGATCTTACTCGCAATGTCTG		
N1	PMA-06013-N1f	TCACTTGCTTCCGTTGAGGTCCCACTTGGAATGCAGAAC	DQ095665	328 bp
	PMA-06014-N1r	TAMRA-GGTTTCGGATGTTACAGCGTCACATGCACATTCAGACTCTTG		
N2	PMA-06015-N2f	TCACTTGCTTCCGTTGAGGATAGCATGGTCCAGCTCAAG	CY002114	299 bp
	PMA-06016-N2r	TAMRA-GGTTTCGGATGTTACAGCGTACATGCTGAGCACTTCCTG		
H2	PMA-06019-H2F	TCACTTGCTTCCGTTGAGGCGTCATTCTTCAGGAACATGG	AY633228	229 bp
	PMA-06020-H2R	TAMRA-GGTTTCGGATGTTACAGCGTGGCCTTGTTGCTATTTCWGG		
H4	PMA-06021-H4F	TCACTTGCTTCCGTTGAGGTTGTTAYCCATTTGATGTGCC	CY004939	324 bp
	PMA-06022-H4R	TAMRA-GGTTTCGGATGTTACAGCGTGTRACTCTTCCAGGGTTGTT		
H8	PMA-06023-H8F	TCACTTGCTTCCGTTGAGGAAGGTTGGTCATACATAGTGG	CY005970	444 bp
	PMA-06024-H8R	TAMRA-GGTTTCGGATGTTACAGCGTGTCCTCTTACTAATGGTCTGG		
H10	PMA-06025-H10F	TCACTTGCTTCCGTTGAGGGATTGACAAGATAAGCACCGG	CY006000	435 bp
	PMA-06026-H10R	TAMRA-GGTTTCGGATGTTACAGCGTTTACTYACTCTACTAGGTGCTAT		
H11	PMA-06027-H11F	TCACTTGCTTCCGTTGAGGACTTAGAAATGTCCCAGCAA	DQ080993	437 bp
	PMA-06028-H11R	TAMRA-GGTTTCGGATGTTACAGCGTCATTTCCCTCGTCTTTGGC		
H12	PMA-06035-H12F	TCACTTGCTTCCGTTGAGGAGTACAAGAACACCAGAGATT	CY006008	537 bp
	PMA-06036-H12R	TAMRA-GGTTTCGGATGTTACAGCGTCTGGCCATCCGCCTTCTAT		
H13	PMA-06029-H13F	TCACTTGCTTCCGTTGAGGGACCCTTCTGCTCCTCATG	AY684886	474 bp
	PMA-06030-H13R	TAMRA-GGTTTCGGATGTTACAGCGTGAAACTGATTGATTCCCCTGG		
H14	PMA-06037-H14F	TCACTTGCTTCCGTTGAGGTCTCCCGACTAAACTGGCTA	M35997	247 bp
	PMA-06038-H14R	TAMRA-GGTTTCGGATGTTACAGCGTCTGCCGCTCTGATTCCTTAC		
H15	PMA-06031-H15F	TCACTTGCTTCCGTTGAGGGACTCCTTGACTGAGATCTGG	CY006032	305 bp
	PMA-06032-H15R	TAMRA-GGTTTCGGATGTTACAGCGTAGTATCACATCTTTGTACCCAC		
H16	PMA-06033-H16F	TCACTTGCTTCCGTTGAGGTAAACTTCTCGTGCTAATCG	AY684888	252 bp
	PMA-06034-H16R	TAMRA-GGTTTCGGATGTTACAGCGTGTCTTCAACTTGATCCCTTC		
N3	PMA-06049-N3F	TCACTTGCTTCCGTTGAGGGGGAAAGARTGGATGCATGT	AY611526	366 bp
	PMA-06050-N3R	TAMRA-GGTTTCGGATGTTACAGCGTGTTGTTGATTCTCATCCAAGG		
N4	PMA-06039-N4F	TCACTTGCTTCCGTTGAGGGGAAGCAATCGACCATGGAT	CY005359	260 bp
	PMA-06040-N4R	TAMRA-GGTTTCGGATGTTACAGCGTCGACACCCATCCATTAGCAT		
N5	PMA-06051-N5F	TCACTTGCTTCCGTTGAGGACTGTTATTGGGTAATGACG	CY004340	459 bp
	PMA-06052-N5R	TAMRA-GGTTTCGGATGTTACAGCGTTGCTTGTTTTGGTCCAACCG		
N6	PMA-06041-N6F	TCACTTGCTTCCGTTGAGGACCTAATAACAATGCTTCGG	AF285887	246 bp
	PMA-06042-N6R	TAMRA-GGTTTCGGATGTTACAGCGTCACTCTTCTATATGCTGTGC		
N7	PMA-06043-N7F	TCACTTGCTTCCGTTGAGGTGTGCAGAGATAAYTGGCA	AJ620349	352 bp
	PMA-06044-N7R	TAMRA-GGTTTCGGATGTTACAGCGTCCGGAATAGCCTGACCAATT		
N8	PMA-06045-N8F	TCACTTGCTTCCGTTGAGGGGGCAMTGATGTATGGATGG	AY300948	340 bp
	PMA-06046-N8R	TAMRA-GGTTTCGGATGTTACAGCGTAAGAATAGCTCCATCGTGCC		
N9	PMA-06047-N9F	TCACTTGCTTCCGTTGAGGTTCTATGCTCTCAGCCAAGG	CY005332	310 bp
	PMA-06048-N9R	TAMRA-GGTTTCGGATGTTACAGCGTTGGCATACGCATTCAGATTC		

Note: Y = (C, T), W = (A, T), R = (A, G), M = (A, C), TAMRA = 6-carboxytetramethylrhodamine.