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Evaluation of the Pan Influenza detection kit utilizing the PLEX-ID and influenza samples from the 2011 respiratory season

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Abstract

A comparison study was performed between the PLEX-ID and the CDC RT-PCR method for the detection and identification of Influenza A viruses using nasopharyngeal samples ($N = 75$) collected between January and May 2011. Overall agreement was 89.3% (67/75 kappa = 0.57 95% CI 0.3–0.89). Positive percent agreement was 92.3% (60/65); negative percent agreement was 70% (7/10). H1N1 pdm09 identified: 42.6% (32/75) and 54.7% (41/75) by PLEX-ID and CDC RT-PCR, respectively. H3N2 identified: 29.3% (22/75) and 32% (24/75) of samples by PLEX-ID and CDC RT-PCR, respectively. Negatives identified: 16% (12/75) and 13.3% (10/75), by PLEX-ID and CDC RT-PCR respectively. For influenza viruses identified as H1N1 pdm09, Influenza A virus A/NEW YORK/15/2009(H1N1 pdm09) was the most prevalent genotype at 50% (16/32), followed by A/CALIFORNIA/05/2009(H1N1 pdm09) at 18.2% (6/32). Updated assay plates containing additional primers designed for H1N1 pdm09 HA and NA genes were utilized for this evaluation. Among H1N1 pdm09 samples, the HA gene was conserved in 96.9% (31/32) of samples. The NA gene was conserved in 96.9% (31/32).

Keywords

Influenza A virus; Genotyping; PLEX-ID; H1N1 pdm09

With regards to H3N2, A/Thailand/CU-B1697/2009 was the most prevalent genotype at 37.5% (9/24) followed by A/Kentucky/UR07-0148/2008 at 29.2% (7/24). For H3N2 samples, the NP and M genes appeared diverse with one deviation in NP accounting for 40.1% (9/22) of the NP base composition and M deviation accounting for 31.8% (7/22) of the M base composition. When examining the overall and positive percent agreement the PLEX-ID performed well compared to the CDC RT-PCR method.

The PLEX-ID platform has shown previously to be a useful tool for respiratory virus surveillance and Influenza A virus detection, identification and genotyping (Chen et al., 2011a,b; Deyde et al., 2010; Forman et al., 2012; Tang et al., 2013; Jeng et al., 2012; Sampath et al., 2007a,b). Updated assay plates including hemagglutinin (HA) and neuraminidase (NA) primers were developed recently to comply with recommendations from regulatory agencies and to provide an immediate characterization of H1N1 pdm09.

The H primer pair was designed to amplify the H1 hemagglutinin to the exception of the other H types, and will therefore amplify both seasonal H1N1 and H1N1 pdm09 (along with other H1 types, e.g. H1N2). The resulting amplicons will distinguish seasonal H1N1 from H1N1pdm09. The N primer pair was designed to specifically amplify a 4-nt fragment of the N1 neuraminidase of H1N1 pdm2009, meaning that generation of an amplicon by this primer pair is in itself already indicative of H1N1 pdm09 with only two minor variants of the expected amplicon have been encountered. Taken together, those two primer pairs thus allow the detection of H1 types and more specifically the characterization of H1N1 pdm09. The performance of these plates were compared to the CDC RT-PCR protocol (<http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol20090428.pdf>) for genotyping circulating influenza strains from the January–May, 2011 influenza season in Baltimore MD.

Nasopharyngeal samples were obtained from patients with influenza like symptoms who were hospitalized at Johns Hopkins Hospital (JHH) between the months of January and May 2011 ($N = 75$). Influenza A positives by DFA, Luminex xTAG RVP1 or RVP Fast (Luminex Corporation, Austin, TX) were sent to the Maryland State Health Laboratory for analysis with the CDC RT-PCR typing method. The CDC RT-PCR typing method was performed as described previously (<http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol20090428.pdf>), and a separate aliquot of each positive sample was de-identified and RNA extraction was performed utilizing the Arrow and its viral NA extraction kit (NorDiag, Inc., Oslo, Norway) prior to amplification and analysis with the PLEX-ID (Abbott Molecular, Des Plaines, IL). Nucleic acid extraction, amplification and analysis were performed according to manufacturer specifications. No access to gold standard results was obtained until after PLEX-ID analysis was complete. This study was approved by the Johns Hopkins University Institutional Review Board.

Overall agreement between the two methods was 89.3% (67/75, kappa = 0.57, 0.3–0.89) while percent agreement for any positive was 92.3% (60/65) with a negative percent agreement of 70% (7/10). The PLEX-ID and the CDC RT-PCR protocol identified 42.7% (32/75) and 54.7% (41/75) of samples as H1N1 pdm09 respectively. The PLEX-ID identified 29.3% (22/75) as H3N2, while the CDC RT-PCR identified 32% (24/77) as H3N2. The PLEX-ID and CDC RT-PCR identified 16% (12/75) and 13.3% (10/75) as negative respectively. For samples that were identified as H1N1, the PLEX-ID identified 6.6% (5/75) as H1N1 of unknown origin, and 2.7% (2/75) as mixed H1N1 genotypes, meaning that the PLEX-ID identified more than one genotype of H1N1 in the same sample. This data is summarized in Table 1.

The genotypes that were identified for Influenza A H1N1 pdm09 are summarized in Table 2a, where the identified H1N1 pdm09 ranged over 9 different genotypes, including samples that were identified as “mixed genotype”. Influenza A virus A/NEW YORK/15/2009(H1N1 pdm09) was the most prevalent genotype identified at 48.5% (16/32), followed by A/CALIFORNIA/05/2009(H1N1 pdm09) at 18.2% (6/32). The samples classified as “mixed genotype” were identified as Influenza A virus A/Mexico City/WRAIR1773T/2010(H1N1) or Influenza A virus A/California/05/2009(H1N1pdm09). For two of the H1N1 of unknown origin samples, the PLEX-ID inferred the following identifications: Influenza A virus A/DISTRICT OF COLUMBIA/INS21/2009(H1N1) and Influenza A virus A/CALIFORNIA/VRDL4/2010(H1N1) (Table 2a).

The detected Influenza A genotypes for samples identified as H3N2 are summarized in Table 2b, and ranged over 10 genotypes, where A/Thailand/CUB1697/2009 was most prevalent at 40.9% (9/22) followed by A/Kentucky/UR07-0148/2008 at 31.8% (7/25). Other H3N2 identifications are also summarized in Table 2b.

In this study, the Pan Influenza typing kit for the PLEX-ID detected Influenza A virus in 92.3% (60/65) of the samples that tested positive via the CDC RT-PCR reference assay. These results are similar to results reported in previous studies (Chen et al., 2011a,b; Deyde et al., 2010). Unidentified samples were likely the result of either low sample quantity or poor quality RNA.

In this study, the initial reported positive percent agreement for H1N1 pdm09 identification was 78% (32/41), which is lower than previously reported (Chen et al., 2011a,b; Deyde et al., 2010). This reported positive percent agreement was impacted by 4 H1N1 detections that were identified as unknown origin, and 2 detections of H1N1 mixed genotype versus the CDC RT-PCR as H1N1 pdm09, although the samples were correctly identified as H1N1, the genotype identified by the PLEX-ID did not match the CDC identification. If the positive percent agreement were resolved to include all H1N1 identifications, it would be 92.7% (38/41) which is closer to previous reports (Chen et al., 2011a,b; Deyde et al., 2010; Tang et al., 2013).

These samples had base compositions within the HA region that differed from H1N1 pdm09. Swine origin was not identified in these samples, and the HA change is the likely reason. As a comparison, in samples identified as H1N1 pdm09 the HA gene was conserved in 96.9% (31/32) and the NA gene was conserved in 96.9% (31/32). The “mixed genotype” viruses had PB2 base compositions that differed from the H1N1 pdm09 samples. This shared deviation may be why the PLEX-ID could not identify clearly one specific genotype in these samples.

In a previous study examining H1N1 pdm09 samples from April–June 2009 and October 2009–January 2011, it was observed that the most prevalent base-pair compositions for each gene primer had remained mostly similar (Jeng et al., 2012). In this current study, the specific ratios of some base pair compositions within certain genes did change based on PLEX-ID analysis. This data suggested the ratio of base compositions within the NS1 gene fluctuated over the testing periods. The results also suggest that the PA base composition may be becoming more diverse due to the decrease in prevalence of the [A36 G25 C27 T24] base compositions over three test periods (Jeng et al., 2012).

The 2010–2011 influenza season differed from the 2009–2010 season because H1N1 pdm09 coexisted with H3N2. A 2012 report illustrated the emergence of novel H3N2 viruses that contain NP, M and NS genes from H1N1 pdm09 (Liu et al., 2012 CDC MMWR 2012). In Table 2b, the NP and M genes of H3N2 appear diverse with one deviation in NP [A31 G25 C21 T24] accounting for 40.1% (9/22) of the NP base composition and a deviation of M [A24 G30 C23 T28] accounting for 29.2% (7/24) of the M base composition. Further analysis of these samples by whole genome sequencing could be useful to determine if there is any relationship between these diverse base compositions and H1N1 pdm09.

The two main limitations of this study are the small number of samples tested ($N = 75$), and the limitations of this technology as PCR products utilized for PLEX-ID analysis do not cover the entire gene for each target. The consequence for this primer design is that certain mutations may go undetected.

Ideally, future studies would be prospective, include a larger number of samples for analysis as well as samples from different US and international regions to examine the geographic distribution of different strains of influenza viruses. Furthermore, additional comparisons between this technology and whole genome sequencing are necessary to evaluate the capability of the PLEX-ID to effectively genotype Influenza A viruses for genotype determination.

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References

- CDC protocol of realtime RTPCR for swine influenza A (H1N1) 28 April 2009 revision. Apr 30. 2009 <http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol20090428.pdf>
- Chen KF, Blyn L, Rothman RE, Rhamachandran P, Valsamakis A, Ecker D, Sampath R, Gaydos CA. RT-PCR and electrospray ionization mass spectrometry (RTPCR/ESI-MS) for identifying acute viral upper respiratory tract infections. *Diagn Microbiol Infect Dis*. 2011a; 69:179–186. [PubMed: 21251562]
- Chen KF, Rothman RE, Ramachandran P, Blyn LB, Sampath R, Ecker DJ, Valsamakis A, Gaydos CA. Rapid Identification viruses from nasal pharyngeal aspirates in acute viral respiratory infections by RTPCR and electrospray ionization mass spectrometry. *J Virol Methods*. 2011b; 173:60–66. [PubMed: 21256867]
- Deyde, VM.; Sampath, R.; Garten, RJ.; Blair, PJ.; Myers, CA.; Massire, C.; Matthews, H.; Svoboda, P.; Reed, MS.; Pohl, J.; Klimov, AI.; Gubareva, LV. Genomic signature-based identification of influenza A viruses using RT-PCR/electrospray ionization mass spectrometry (ESI-MS) technology; PLoS ONE. 2010. p. 5 www.plosone.org
- Forman MS, Avanti S, Newman C, Gaydos CA, Milstone AM, Valsamakis A. Diagnostic performance of two highly multiplexed respiratory virus assays in a pediatric cohort. *J Clin Virol*. 2012; 55:168–172. [PubMed: 22832060]
- Jeng K, Massire C, Zembower TR, Deyde VM, Gubareva LV, Hsieh YH, Rothman RE, Sampath R, Penugonda S, Metzgar D, Blyn LB, Hardick J, Gaydos CA. Monitoring seasonal influenza A evolution: rapid 2009 pandemic H1N1 surveillance with an reverse transcription polymerase chain reaction/electrospray ionization mass spectrometry assay. *J Clin Virol*. 2012; 54:332–336. [PubMed: 22673129]
- Liu Q, Ma J, Liu H, Qi W, Anderson J, Henry SC, Hesse SC, Richt JA, Maw. Emergence of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in the United States. *Arch Virol*. 2012; 157:555–562. [PubMed: 22198410]
- Sampath, R.; Russell, KL.; Massire, C.; Eshoo, MW.; Harpin, V.; Blyn, LB.; Melton, R.; Ivy, C.; Pennella, T.; Li, F.; Levene, H.; Hall, TA.; Libby, B.; Fan, N.; Walcott, DJ.; Ranken, R.; Pear, M.; Schink, A.; Gutierrez, J.; Drader, J.; Moore, D.; Metzgar, D.; Addington, L.; Rothman, R.; Gaydos, CA.; Yang, S.; George, K.; Fuschino, ME.; Dean, AB.; Stallknecht, DE.; Goekjian, G.; Yingst, S.; Monteville, M.; Saad, MD.; Whitehouse, CA.; Baldwin, C.; Rudnick, KH.; Hofstadler, SA.; Lemon, SM.; Ecker, DJ. Global surveillance of emerging influenza virus genotypes by mass spectrometry; PLoS ONE. 2007a. p. 2 www.plosone.org
- Sampath R, Hall TA, Massire C, Li F, Blyn LB, Eshoo MW, Hofstadler SA, Ecker DJ. Rapid identification of emerging infectious agents using PCR and electrospray ionization mass spectrometry. *Ann NY Acad Sci*. 2007b; 1102:109–120. [PubMed: 17470915]
- Tang YW, Lowery KS, Valsamakis A, Schaefer VC, Chappell JD, White-Abell J, Quinn CD, Li H, Washington CA, Cromwell J, Giamanco CM, Forman M, Holden J, Rothman RE, Parker ML, Ortenberg EV, Zhang L, Lin YL, Gaydos CA. Validation of a PLEX-ID flu device for simultaneous detection and identification of influenza viruses A and B. *J Clin Microbiol*. 2013; 51:40–45. [PubMed: 23077123]

Table 1

Data summary for sample identification of PLEX ID compared to CDC RT-PCR gold standard.

Component	+ CDC ^a /+ PLEX-ID	- CDC/- PLEX-ID	+ CDC/- PLEX-ID	- CDC/+ PLEX-ID	NA CDC/+ PLEX-ID	Percent agreement (N, kappa, 95% CI)
All positives	60	NA ^b	5	3	NA	92.3% (60/65)
Negatives	NA	7	0	0	NA	70% (7/10)
Total	60	7	5	3	NA	89.3% (67/75 0.57, 0.30-0.89)
Initial H1N1 pdm09 positives ^c	32	NA	3	1	6	78% (32/41)
Resolved All H1N1 positives ^d	38	NA	NA		0	92.7% (38/41)
H3N2 positives	22	NA	2	2	NA	91.7% (22/24)

^a CDC RT-PCR method.

^b Not applicable.

^c Calculated without inclusion of 6 samples identified as H1N1 of unknown origin or H1N1 mixed genotype as the CDC RT-PCR method does not perform these analyses.

^d Calculated with inclusion of 6 samples identified as H1N1 of unknown origin or H1N1 mixed genotype.

Table 2a

Plex ID genomic prints and inferred strain for samples identified as H1N1 pdm09 by CDC RT PCR protocol. Strains were collected from January to May 2011. Boxes shaded in light gray represent one base pair change from A/California/04/2009 reference strain while dark gray represent two base pair changes.

Number of unique samples	Plex ID	viral strain	Influenza A	PB1	NP	M1	PA	NS1	PB2	HA	NA
16	NEW YORK/15/2009	(Pandemic-H1N1)		A39 G32 C24 T33 [0000]	A35 G21 C20 T25 [0000]	A24 G28 C24 T29 [0000]	A36 C25 C27 T24 [0000]	A36 G35 C20 T28 [0000]	A41 G24 C22 T17 [0000]	A33 G33 C19 T18 [0000]	A14 G12 C13 T19 [0000]
3	NEW YORK/4057/2009	(Pandemic-H1N1)-NS1-S1 SNP		A39 G32 C24 T33 [0000]	A35 G21 C20 T25 [0000]	A24 G28 C24 T29 [0000]	A36 C25 C27 T24 [0000]	A37 G34 C20 T28 [+1 -1 00] [0000]	A41 G24 C22 T17 [0000]	A33 G33 C19 T18 [0000]	A14 G12 C13 T19 [0000]
3	SANTO DOMINGO/WR1068N/2009	(Pandemic-H1N1)-PB1-S4 SNP		A39 G32 C25 T32 [00+1-1]	A35 G21 C20 T25 [0000]	A24 G28 C24 T29 [0000]	A36 C25 C27 T24 [0000]	A36 G35 C20 T28 [0000]	A41 G24 C22 T17 [0000]	A33 G33 C19 T18 [0000]	A14 G12 C13 T19 [0000]
1	SINGAPORE/GP2711/2009	(Pandemic-H1N1)-NUC-S2 SNP		A39 G32 C24 T33 [0000]	A34 G22 C20 T25 [-1 +1 00]	A24 G28 C24 T29 [0000]	A35 G26 C27 T24 [-1 +1 00]	A36 G35 C20 T28 [0000]	A41 G24 C22 T17 [0000]	A33 G33 C19 T18 [0000]	A14 G12 C13 T19 [0000]
1	WISCONSIN/629-D00698/2009	(Pandemic-H1N1)-HA-S1 SNP		A39 G32 C24 T33 [0000]	A35 G21 C20 T25 [0000]	A24 G28 C24 T29 [0000]	A36 C25 C27 T24 [0000]	A36 G35 C20 T28 [0000]	A41 G24 C22 T17 [0000]	A34 G32 C19 T18 [+1 -1 00]	A14 G12 C13 T19 [0000]
1	HIROSHIMA/220/2009	(Pandemic-H1N1)		A39 G32 C24 T33 [0000]	A35 G21 C20 T25 [0000]	A24 G28 C24 T29 [0000]	A36 C25 C27 T24 [0000]	- [00 -1 +1] [0000]	A42 G23 C22 T17 [+1 -1 00]	A33 G33 C19 T18 [0000]	- A14 G12 C13 T19 [0000]
1	JAPAN/PR1070/2009	(Pandemic-H1N1)-PA-S3 SNP		A39 G32 C24 T33 [0000]	A35 G21 C20 T25 [0000]	A24 G28 C24 T29 [0000]	A36 G25 C26 T25 [00 -1 +1]	A36 G35 C20 T28 [0000]	A41 G24 C22 T17 [0000]	A33 G33 C19 T18 [0000]	A14 G12 C13 T19 [0000]
6	CALIFORNIA/05/2009	(Pandemic-H1N1)		A39 G32 C24 T33 [0000]	A35 G21 C20 T25 [0000]	A24 G28 C24 T29 [0000]	A35 G26 C27 T24 [0000]	A37 G34 C20 T28 [0000]	A41 G24 C22 T17 [0000]	A33 G33 C19 T18 [0000]	A14 G12 C13 T19 [0000]

Number of unique samples	Plex ID	viral strain	Influenza A	PB1	NP	M1	PA	NS1	PB2	HA	NA
1		DISTRICT OF COLUMBIA/INS21/2009(H1N1)		[0000]	[0000]	[0000]	[-1 +1 00]	[+1 -1 00]	[0000]	[0000]	[0000]
				-				-			
				A39 G32	A35 G21	A24 G28	A36 C25	A36 G34	A41 G24	A33 G33	A14 G12
				C24 T33	C20 T25	C24 T29	C27 T24	C20 T29	C22 T17	C18 T19	C13 T19
				[0000]	[0000]	[0000]	[0000]	[0 -1 0+1]	[0000]	[00-1+1]	[0000]
1		CALIFORNIA/VRDL4/2010(H1N1)		-							
					A35 G21	A24 G28	A35 G26	A36 G35	A41 G24	A33 G33	A14 G12
				C20 T25	C20 T25	C24 T29	C27 T24	C20 T28	C22 T17	C20 T17	C13 T19
					[0000]	[0000]	[-1 +1 00]	[0000]	[0000]	[00+2-2]	[0000]
				A39 G32	A35 G21	A24 G28	A35 G26	A36 G34	A41 G23	A33 G33	A14 G12
2		MEXICO CITY/WRAIR1773T/2010(H1N1) OR CALIFORNIA/05/2009(Pandemic-H1N1)									
				C24 T33	C20 T25	C24 T29	C27 T24	C21 T28	C23 T17	C19 T18	C13 T19
				[0000]	[0000]	[0000]	[-1 +1 00]	[0 -1 +10]	[0 -1 +10]	[0000]	[0000]
								A37 G34			-
								C20 T28			
								[+1 -1 00]			

Plex ID genomic prints and inferred strain for samples identified as H3N2 by CDC RT PCR protocol. Strains were collected from January–May 2011.

Table 2b

Number of unique samples	Plex ID viral strain (Influenza A)	PB1	NP	MI	PA	NS1	PB2
8	THAILAND/CU-B1697/2009(H3N2)	A40 G31 C24 T33	A31 G24 C21 T25	A24 G30 C22 T29	A37 G25 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
1	THAILAND/CU-H16/2009(H3N2)	A40 G31 C24 T33	A31 G24 C21 T25	A24 G30 C22 T29	A37 G24 C27 T24	A39 G31 C21 T28	–
7	KENTUCKY/UR07-0148/2008(H3N2)	A40 G31 C24 T33	A31 G25 C21 T24	A24 G30 C23 T28	A37 G24 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
2	KENTUCKY/UR07-0108/2008(H3N2)	A40 G31 C24 T33	A31 G25 C21 T24	A24 G30 C22 T29	A36 G25 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
1	KENTUCKY/UR07-0109/2008(H3N2)	–	–	A24 G30 C22 T29	A37 G24 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
1	CALIFORNIA/VRDL334/2009(H3N2)	A41 G30 C24 T33	–	A24 G30 C22 T29	A37 G24 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
1	CheongJU/H383/2007(H3N2)	A40 G31 C23 T34	–	A24 G30 C22 T29	A37 G24 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
1	NEW YORK/3079/2009(H3N2)	A41 G30 C24 T33	A31 G25 C21 T24	–	A37 G24 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
1	BOSTON/47/2008(H3N2)	A41 G30 C24 T33	A31 G25 C22 T23	A24 G30 C22 T29	A37 G24 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20
1	TEXAS/AF01/2007(H3N2)	A40 G31 C24 T33	A31 G25 C22 T23	A25 G29 C22 T29	A37 G24 C27 T24	A39 G31 C21 T28	A38 G27 C19 T20