2 Aguilar-Yáñez JM, Portillo-Lara R, Mendoza-Ochoa GI *et al.* An Influenza A/H1N1/2009 hemagglutinin vaccine produced in *Escherichia coli*. PLoS ONE 2010; 5:e11694. DOI: 10.1371/journal.pone.0011694.
3 World Health Organization website. CDC protocol of realtime RT-PCR for influenza A (H1N1). 2009. Available at: http://www.who.int/csr/resources/publications. Accessed May 5, 2009.
4 DuBois RM, Aguilar-Yañez JM, Mendoza-Ochoa GI *et al.* The receptor binding domain of influenza virus hemagglutinin protein produced in *Escherichia coli* folds into its native, immunogenic structure. J Virol 2011; 85:865–872.
5 Igarashi M, Ito K, Yoshida R, Tomabechi D, Kida H, Takada A *et al.* Predicting the antigenic structure of the pandemic (H1N1) 2009 influenza virus hemagglutinin. PLoS ONE 2010; 5:e8553. DOI: 10.1371/journal.pone.0008553.

A monoclonal antibody-based ELISA for differential diagnosis of 2009 pandemic H1N1

Hongxia Shao,[a,b] Jianqiang Ye,[a] Amy L.Vincent,[c] Haichen Song,[d] Danielle Hickman,[a] Aijian Qin,[b] Chinta Lamichhane,[d] Daniel R. Perez[a]

[a]Department of Veterinary Medicine, University of Maryland, College Park and Virginia-Maryland Regional College of Veterinary Medicine, College Park, MD, USA. [b]The Key Laboratory of Jiangsu Preventive Veterinary Medicine, Yangzhou University, Yangzhou, China. [c]Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, USDA-ARS, Ames, IA, USA. [d]Synbiotics Corporation, College Park, MD, USA.

Keywords 2009 pandemic H1N1 virus, ELISA, mAb.

Please cite this paper as: Shao *et al.* (2011) A monoclonal antibody-based ELISA for differential diagnosis of 2009 pandemic H1N1. Influenza and Other Respiratory Viruses 5 (Suppl. 1), 132–158.

Abstract

The swine-origin 2009 pandemic H1N1 virus (pdmH1N1) is genetically related to North American swine H1 influenza viruses and unrelated to human seasonal H1 viruses. Currently, specific diagnosis of pdmH1N1 relies on RT-PCR. In order to develop an assay that does not rely in amplification of the viral genome, a conventional sandwich ELISA for detection of the pdmH1N1 was developed. The sandwich ELISA was based on three monoclonal antibodies (3B2, 5H7, and 12F3) against pdmH1N1. 5H7 and 12F3 were selected as capture antibodies and biotin-conjugated 3B2 was subsequently selected as the detection antibody in the ELISA. The results showed the ELISA had high specificity for pdmH1N1 strains and no reaction with other swine H1 viruses, human seasonal H1N1 or H3N2 viruses, or avian influenza viruses. The limit of detection of the ELISA ranged from $3{\cdot}2 \times 10^3$ to $1{\cdot}5 \times 10^4$ $TCID_{50}$/ml. When the ELISA was used to detect viruses in nasal wash samples from infected ferrets, it showed 90·1% sensitivity and 100% specificity compared to the "gold standard" – virus isolation. Our studies highlight a convenient assay for specific diagnosis of the 2009 pandemic H1N1-like viruses.

Introduction

In April 2009, a novel H1N1 influenza virus emerged in North America and caused the first influenza pandemic of the 21st century.[1–4] The 2009 pandemic H1N1 (pdmH1N1) has a unique gene constellation that was not previously identified in any species or elsewhere. It is genetically related to the triple reassortant swine H1N1 influenza viruses currently circulating in North America, with the exception of the neuraminidase (NA) and matrix (M) genes, which are derived from a Eurasian swine influenza virus.

Swine H1N1 influenza viruses were first isolated in 1930 and continued to circulate in North America with very little antigenic changes (classical swine H1N1) until 1998. Since 1999, however, the antigenic make up of swine H1 viruses has shown increased diversity due to multiple reassortment events and the introduction of H1N1 genes from human influenza viruses. Currently, four swine H1 clusters (α, β, γ, δ) are found endemic in the North American swine population.[5,6] These swine H1 viruses show substantial antigenic drift compared to the classical swine H1 viruses. Cluster δ swine H1 is derived from current human H1 viruses, and there is a substantial antigenic divergence between classical swine H1 and human seasonal H1 viruses. Epidemiological evidence shows a two-way transmission of influenza viruses between swine and humans, and such events lead to the emergence of the pdmH1N1 virus.[5,7,8] Phylogenetic analysis have suggested that possible ancestors of the eight genes of pdmH1N1 were circulating in the swine population for at least 10 years prior to the emergence of the pdmH1N1 virus in humans, although the

pdmH1N1 virus itself was not isolated from pigs until after the pandemic. Interestingly, pdmH1N1 infections have been reported not only in humans and pigs, but also in other animal species such as turkeys, cats, ferrets, cheetahs, and dogs.[9–11] After the first report of pdmH1N1 infection in swine in Canada, other countries, including Argentina, Australia, Singapore, Northern Ireland, Finland, Iceland, England, United States, Japan, and China reported outbreaks of pdmH1N1 in swine as well.[9,12–14] The ample geographic range of pdmH1N1 outbreaks in swine, its apparent broad host range, and the possibility of two-way transmission between swine and humans poses a tremendous challenge for controlling the virus. Therefore, to differentiate pdmH1N1 from other H1 strains, particularly in swine and human populations, is an important issue to ascertain the magnitude of the disease caused by the pdmH1N1. In this study, we developed an ELISA assay to discriminate pdmH1N1 strains from other swine and human H1 viruses.

Materials and methods

Viruses, cells, and antibodies

Madin-Darby canine kidney (MDCK) cells (ATCC, Manassas, VA, USA) were maintained in modified Eagle's medium (MEM) containing 5% FBS. A/California/04/09/H1N1 virus (Ca/04) was kindly provided by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. Other viruses are listed in Table 1. Viruses were propagated in MDCK cells and stored at −70°C until use. Viruses were titrated by the Reed and Muench method to determine the median tissue culture infectious dose ($TCID_{50}$).[15] Three monoclonal antibodies (3B2, 5H7, and 12F3) against HA of 2009 pandemic H1N1 were prepared in our laboratory following previously described methods (Shao and Perez *et al.*, unpublished).

Purification and labeling of mAbs

mAb 3B2, 5H7 and 12F3 were purified on a Protein G–Sepharose affinity column (Upstate Biotechnology, Lake Placid, NY, USA). Biotinylation of the detection antibody in the ELISA was performed using Sulfo-NHS-LC-biotin (sulfosuccinimidyl-6-(biotinamido)hexanoate; Pierce, Rockford, IL, USA) according to the manufacturer's instructions.

Sandwich ELISA

Purified 5H7 and 12F3 were selected as the capture antibody, and biotin-conjugated 3B2 was selected as the detection antibody, and HRP-conjugated streptavidin (Abcom, Cambridge, MA, USA) was developed using the TMB substrate system (KPL, Gaithersburg, MD, USA). In brief, the mixture of the purified 5H7 and 12F3 (2·0 and 2·2 μg/ml respectively, in carbonate/bicarbonate buffer, pH 9·6) was coated to 96-well plates (test well, T) for 12 h at 4°C. At the same time, a control antibody was coated to 96-well plates (control well, C). After blocking the plates with 5% (w/v) non-fat milk in PBS for 1 hour at 37°C, the samples were diluted in extract buffer (1%Tween-20, 0·5%BSA in PBS) and added to the wells (100 μl/well, each sample was

Table 1. Specificity assay of the sandwich ELISA

Virus	Result (T/C)
A/Bristane/59/2007(H1N1)	−0·8
A/Fort Monmouth/1/1947(H1N1)	−1·2
A/PR8/34(H1N1)	−1·2
A/NewCaledonia/20/99(H1N1)	−1·1
A/Malaya/302/1954(H1N1)	−1·2
A/WSN/1933(H1N1)	−0·9
A/mallard/New York/6750/78(H2N2)	−1·1
A/Brisbane/10/2007(H3N2)	−1·2
A/duck/Hongkong/3/75(H3N2)	−1·1
A/Viet Nam/1203/2004(H5N1)	−1·0
A/mallard/Alberta/206/96(H6N8)	−0·7
A/chicken/Delaware/VIVA/2004(H7N2)	−1·0
A/mallard/Alberta/194/92(H8N4)	−1·0
A/guinea fowl/Hong Kong/WF10/99(H9N2)	−0·9
A/pintail/Alberta/202/00(H10N7)	−0·8
A/duck/Maryland/2T70/2004(H11N9)	−0·9
A/mallard/Alberta/238/96(H12N5)	−0·8
A/mallard/Alberta/146/2001(H13N6)	−1·1
A/swine/Minnesota/02053/2008(H1N1) (α)	−0·9
A/swine/Minnesota/02093/2008(H1N1) (α)	−1·0
A/swine/North Carolina/02084/200(H1N1) (β)	−0·8
A/swine/Kentucky/02086/2008(H1N1) (β)	−1·2
A/swine/Nebraska/02013/2008(H1N1) (β)	−0·8
A/swine/Ohio/02026/2008(H1N1) (γ)	−0·8
A/swine/Missouri/02060/2008(H1N1) (γ)	−0·7
A/swine/Iowa/02096/2008(H1N1) (γ)	−1·2
A/swine/Ohio/511445/2007(H1N1) (γ)	−0·7
A/swine/North Carolina/02023/2008(H1N1) (γ)	−0·9
A/swine/Texas/01976/2008(H1N2) (δ)	−0·8
A/swine/Iowa/02039/2008(H1N2) (δ)	−0·9
A/swine/Minnesota/02011/2008(H1N2) (δ)	−0·7
A/swine/Iowa/15/30(H1N1) (classical)	−0·8
A/swine/Tennessee/25/77(H1N1) (classical)	−1·1
A/Netherlands/602(H1N1pdm)	+11
A/California//04/2009(H1N1pdm)	+4·2
A/Mexico/4108/2009(H1N1pdm)	+10
Rg-NY/18HA1: 7NL/602(H1N1pdm)*	+12
Rg-D225G-HA-NL/602(H1N1pdm)†	+3·5

*Rg-NY/18HA1:7NL/602, reverse genetic recombinant carrying the HA gene from A/New York/18/2009 (H1N1) and remaining seven genes from A/Netherland/602/2009(H1N1).

†Rg-D225G-HA-NL/602 (kindly provided by Dr Ron A. M. Fouchier), reverse genetic A/Netherland/602/2009(H1N1) virus carrying the HA gene containing D225G mutation.

added to four wells–two for T wells and two for C wells–and the mixture was incubated at 37°C for 1 hour. After four washes, 100 μl biotin-conjugated 3B2 (0·25 μg/ml) in dilution buffer (0·5% BSA in PBS) was added to the wells and the mixture was incubated for 1 h at 37°C. Following three washes, 100 μl diluted HRP-conjugated streptavidin (62·5 ng/ml) in dilution buffer was added to the plates. After incubation for 1 h at 37°C, the plates were washed five times, and the binding developed using the TMB substrate system for 30 minutes. The ratio of the average OD_{650} value of the T wells to that of the C wells (T/C) of individual samples was calculated. T/C values >1·5 were considered positive in the sandwich ELISA.

Results

Specificity of sandwich ELISA for pdmH1N1 detection

We developed three monoclonal antibodies, 3B2, 5H7, and 12F3, against a prototypical pdmH1N1 strain, A/California/04/2009 (H1N1) (Ca/04). These monoclonals were used to develop a rapid sandwich ELISA for specific diagnosis of pdmH1N1 strains. Purified 5H7 and 12F3 were used as capture antibodies, whereas the biotin-conjugated 3B2 was used as detection antibody. The sandwich ELISA showed strong reaction with different pdmH1N1 strains as described in Table 1. The T/C ratios of Ca/04, A/Netherland/602/2009 (H1N1) (NL/602), A/Mexico/4108/2009 (H1N1), and A/New York/18/2009 (H1N1) were 11, 10, 4·2, and 12, respectively, which are higher than the cut off value of 1·5.

In order to evaluate if the sandwich ELISA could distinguish the pdmH1N1 from other swine H1 clusters (α, β, γ, δ), 14 swine influenza strains spanning these clusters were tested. These viruses were first diluted 1:10 in extract buffer, and then added to the coated plates. As shown in Table 1, the T/C ratios of these viruses were <1·5, and therefore showed negative ELISA result. Likewise, testing of human seasonal virus strains A/Brisbane/59/2007 (H1N1), A/FM/1/1947 (H1N1), A/PR8/1934 (H1N1), A/NewCaledonia/20/99 (H1N1), A/Malaya/302/1954(H1N1), A/WSN/1933 (H1N1), and A/Brisbane/10/2007 (H3N2) also showed negative ELISA results. Furthermore, the sandwich ELISA showed no cross reaction with avian influenza viruses, including strains of the H2, H3, H5, H6, H7, H8, H9, H10, H11, H12, and H13 subtypes.

More recently, the mutation D222G in the HA of some pdmH1N1 strains has been associated with exacerbated disease and altered receptor binding.[16–20] To evaluate if such mutant could be detected in our sandwich ELISA, we tested a mutant of A/Netherland/602/2009 (H1N1) carrying the D222G mutation (engineered by reverse genetics). As described in Table 1, our ELISA could still capture the D222G mutant virus and showed a positive reaction, which highlights the specificity of our assay for pdmH1N1 strains, even those with mutations.

Limit of the detection of the pdmH1N1 sandwich ELISA

To evaluate the sensitivity of the ELISA, we used the serially diluted pdmH1N1 viruses to determine the limit of detection (LOD). As shown in Table 2, in our ELISA the highest positive dilutions of NL/602 and Ca/04 were 1:320 and 1:160, respectively. The LOD of the sandwich ELISA by $TCID_{50}$ was $3{\cdot}2 \times 10^3$ and $1{\cdot}5 \times 10^4$ $TCID_{50}$/ml, for NL/602 and Ca/04, respectively. It is important to note that the T/C ratio from NL/602 and Ca/04 viruses showed clearly a dose dependent effect, while the T/C ratio of A/swine/Iowa/30 (H1N1) did not show the same dependence and was always <1·5, corroborating the high specificity of the sandwich ELISA for pdmH1N1 strains. Although we did not compare our ELISA with other current commercial rapid influenza detection kits, the LOD of our ELISA assay is similar to other commercial kits that detect human seasonal influenza virus.[21]

Comparison of the sandwich ELISA with the "gold standard" – virus isolation

In order to further evaluate the feasibility of the application of the ELISA to clinical samples, 70 nasal wash samples

Table 2. Limit of detection (LOD) of the sandwich ELISA

Virus	Titer ($TCID_{50}$/ml)	Result (T/C) at different dilution								LOD (TCID50/ml)
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	
NL/602 (H1N1)	5 × 10^6	+(11)	+(10)	+(5·4)	+(3·8)	+(1·9)	+(1·7)	−(1·4)	−(1·0)	1·5 × 10^4
Ca/04 (H1N1)	5·032 × 10^5	+(8·0)	+(4·8)	+(3·6)	+(2·1)	+(1·6)	−(1·4)	−(1·1)	−(1·1)	3·2 × 10^3
Sw/IA/30 (H1N1)	1·58 × 10^6	−(0·8)	−(0·8)	−(0·9)	−(1·0)	−(0·9)	−(0·8)	−(1·1)	−(0·9)	–

from ferrets, 56 of those previously infected with Ca/04 and shown positive by virus isolation, were tested. The samples were diluted 1:1 in extract buffer and then tested using the sandwich ELISA. Result showed 51 out of 56 positive samples by virus isolation were positive also by the sandwich ELISA (sensitivity 90·1%). The 14 samples tested that were negative by virus isolation were also negative in the ELISA, indicating 100% specificity for our assay. These results show not only that our ELISA has high compatibility with the virus culture method, but also indicates this application can be used for clinical samples.

Discussion

Although real time RT-PCR targeting the HA gene has been used for specific diagnosis of pdmH1N1 with high sensitivity,[22–27] it is a method that requires manipulation of the sample to extract viral RNA, and it is prone to cross-contamination during the PCR steps. In this study, we described a convenient sandwich ELISA based on three mAbs developed against the pdmH1N1 strain. The ELISA not only shows high specificity for pdmH1N1 strain, but also shows great sensitivity. The ELISA could distinguish pdmH1N1 strains from human seasonal H1 and H3 viruses and, more importantly, from other swine H1 viruses. We must note that current rapid diagnostic tests cannot be used to differentiate pdmH1N1 from swine or human H1 viruses.[28,29] More recently, Miyoshi-Akiyama *et al.* and Miao *et al.* developed an immunochromatographic assay and a indirect immunofluorescence assay, respectively, for specific diagnosis of pdmH1N1.[30,31] Such tests can distinguish pdmH1N1 strains from human seasonal influenza H1N1, however, these tests have not been evaluated to test whether they can distinguish among other swine influenza viruses.

It is also worth noting that the sensitivity of commercial rapid antigen-based diagnostic tests for detecting pdmH1N1 is lower than that for human seasonal influenza viruses.[28,29] A study by Kok *et al.*[32] showed that sensitivity of the current rapid antigenic tests for pdmH1N1 is only 53·4%, whereas that for seasonal influenza A is 74·2%. Chen *et al.*[33] developed a dot-ELISA and increased the sensitivity for influenza rapid antigen detection. However, the dot-ELISA developed by Chen cannot distinguish among subtypes. The LOD of our ELISA is between $3{\cdot}2 \times 10^3$ to $1{\cdot}5 \times 10^4$ TCID50/ml, comparable to the LOD of rapid diagnostic tests for human seasonal influenza viruses.[21] Compared to the "gold standard"–virus isolation–our sandwich ELISA showed 90·1% sensitivity using ferret nasal washes. Our results highlight the potential application of our sandwich ELISA for the specific diagnosis of pdmH1N1 viruses.

Acknowledgements

We are indebted to Yonas Araya, Theresa Wolter, and Ivan Gomez-Osorio for their excellent laboratory techniques and animal handling assistance. We would like to thank Andrea Ferrero for her laboratory managerial skills. This research was possible through funding by the CDC-HHS grant (1U01CI000355), NIAID-NIH grant, (R01AI052155), CSREES-USDA grant (1865-05523), and NIAID-NIH contract (HHSN266186700010C).

References

1 Libster R, Bugna J, Coviello S *et al.* Pediatric hospitalizations associated with 2009 pandemic influenza A (H1N1) in Argentina. N Engl J Med 2010; 362:45–55.
2 Louie JK, Acosta M, Winter K *et al.* Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. JAMA 2009; 302:1896–1902.
3 Donaldson LJ, Rutter PD, Ellis BM *et al.* Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. BMJ 2009; 339:b5213.
4 Garten RJ, Davis CT, Russell CA *et al.* Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 2009; 325:197–201.
5 Vincent AL, Ma W, Lager KM *et al.* Characterization of a newly emerged genetic cluster of H1N1 and H1N2 swine influenza virus in the United States. Virus Genes 2009; 39:176–185.
6 Vincent AL, Lager KM, Faaberg KS *et al.* Experimental inoculation of pigs with pandemic H1N1 2009 virus and HI cross-reactivity with contemporary swine influenza virus antisera. Influenza Other Respi Viruses 2010; 4:53–60.
7 Shinde V, Bridges CB, Uyeki TM *et al.* Triple-reassortant swine influenza A (H1) in humans in the United States, 2005–2009. N Engl J Med 2009; 360:2616–2625.
8 Karasin AI, Carman S, Olsen CW. Identification of human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada (2003 to 2005). J Clin Microbiol 2006; 44:1123–1126.
9 OIE. A/H1N1 Influenza, Canada. Available at: http://www.oie.int/wahis/public.php?page¼single_report&pop¼1&reportid¼8065
10 Weingartl HM, Berhane Y, Hisanaga T *et al.* Genetic and Pathobiologic characterization of pandemic H1N1 2009 influenza viruses from a naturally infected swine herd. J Virol 2010; 84:2245–2256.
11 ProMED. Archive Number 20091202.4111. Available at: http://www.promedmail.org/pls/otn/f?p¼2400:1001:50943::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1004,80318.
12 Pereda A, Cappuccio J, Quiroga MA *et al.* Pandemic (H1N1) 2009 outbreak on pig farm, Argentina. Emerg Infect Dis 2010; 16:304–307.
13 http://www.pighealth.com/influenza.htm.
14 Vijaykrishna D, Poon LLM, Zhu HC *et al.* Reassortment of Pandemic H1N1/2009 Influenza A Virus in Swine. Science 2010; 5985:1529.
15 Reed LJ, Muench H. A simple method for estimating 50 percent endpoints. Am J Hyg 1938; 147:265–274.
16 Miller RR, MacLean AR, Gunson RN *et al.* Occurrence of haemagglutinin mutation D222G in pandemic influenza A(H1N1) infected patients in the West of Scotland, United Kingdom, 2009–10. Euro Surveill 2010; 15:19546.

17 Mak GC, Au KW, Tai LS *et al.* Association of D222G substitution in haemagglutinin of 2009 pandemic influenza A (H1N1) with severe disease. Euro Surveill 2010; 15:19534.

18 Potdar VA, Chadha MS, Jadhav SM *et al.* Genetic characterization of the influenza A pandemic (H1N1) 2009 virus isolates from India. PLoS ONE 2010; 5:e9693.

19 Kilander A, Rykkvin R, Dudman SG *et al.* Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009-2010. Euro Surveill 2010; 15:19498.

20 World Health Organization. Preliminary review of D222G amino acid substitution in the haemagglutinin of pandemic influenza A (H1N1) 2009 viruses. Wkly Epidemiol Rec 2010; 85:21–22.

21 http://www.fda.gov/MedicalDevices/Safety/AlertsandNotices/TipsandArticlesonDeviceSafety/ucm109385.htm.

22 http://www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf

23 Ginocchio CC, Zhang F, Manji R *et al.* Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J Clin Virol 2009; 45:191–195.

24 Poon LL, Chan KH, Smith GJ *et al.* Molecular detection of a novel human influenza (H1N1) of pandemic potential by conventional and real-time quantitative RT-PCR assays. Clin Chem 2009; 55:1555–1558.

25 Wang R, Sheng ZM, Taubenberger JK. Detection of novel (swine origin) H1N1 influenza A virus by quantitative real-time reverse transcription-PCR. J Clin Microbiol 2009; 47:2675–2677.

26 Wenzel JJ, Walch H, Bollwein M *et al.* Library of prefabricated locked nucleic acid hydrolysis probes facilitates rapid development of reverse-transcription quantitative real-time PCR assays for detection of novel influenza A/H1N1/09 virus. Clin Chem 2009; 55:2218–2222.

27 Hui Dong Y, Xiong H, Yan A *et al.* Detection of human novel influenza A(H1N1) viruses using multi-fluorescent real-time RT-PCR. Virus Res 2010; 147:85–90.

28 Uyeki TM, Prasad R, Vukotich C *et al.* Low sensitivity of rapid diagnostic test for influenza. Clin Infect Dis 2009; 48:e89–e92.

29 Drexler JF, Helmer A, Kirberg H *et al.* Poor clinical sensitivity of rapid antigen test for influenza A pandemic (H1N1) 2009 virus. Emerg Infect Dis 2009; 15:1662–1664.

30 Miyoshi-Akiyama T, Narahara K, Mori S *et al.* Development of an immunochromatographic assay specifically detecting pandemic H1N1 (2009) influenza virus. J Clin Microbiol 2010; 48:703–708.

31 Higgins AD, Shaw CJ, Johnson JG *et al.* A Monoclonal Antibody Kit for Identification of the Novel 2009 H1N1 Influenza A Virus. J Clin Microbiol 2010; 48:2677–2682.

32 Kok J, Blyth CC, Foo H *et al.* Comparison of a Rapid Antigen Test with Nucleic Acid Testing during Cocirculation of Pandemic Influenza A/H1N1 2009 and Seasonal Influenza A/H3N2. J Clin Microbiol 2010; 48:290–294.

33 Chen Y, Xu F, Gui X *et al.* A rapid test for the detection of influenza A virus including pandemic influenza A/H1N1 2009. J Virol Methods 2010; 167:100–102.

Results of influenza virus detection using different methods

Sosorbaramyn Tsatsral,[a] Tsedenbalyn Naranzul,[a] Nyamdavaagyn Naranbold,[a] Dashdondogyn Enkhsaikhan,[a] Chuluunbaataryn Maitsetseg,[a] Gungerjavyn Nyamaa,[a] Badarchyn Darmaa,[a] Pagbajabyn Nymadawa[a,b]

[a]National Influenza Center, National Center of Communicable Disease, Ulaanbaatar, Mongolia. [b]Mongolian Academy of Medical Sciences, Ulaanbaatar, Mongolia.

Keywords Direct immune-fluorescent-microscopy assay, influenza and influenza-like illness, Influenza sentinel surveillance sites, MDCK cell culture, rt RT-PCR.

Please cite this paper as: Tsatsral *et al.* (2011) Results of influenza virus detection using different methods. Influenza and Other Respiratory Viruses 5 (Suppl. 1), 132–158.

Introduction

The timely and reliable laboratory evidences are vital factors for field epidemiologists trying to control outbreaks of infectious diseases and for the practicing clinicians to properly manage disease cases. Therefore, analysis of new detection methods in comparison to the routine "classical" methods is essential to select new methods to be introduced into health service practices, especially in developing countries. In this study we have compared rt-RT-PCR detection of influenza viruses and direct fluorescent-antibody assay using R-Mix hybrid cells (A549&Mv1Lu) with the "classical" cell culture methods in developing country settings.

Materials and methods

Clinical samples

In this study, we analyzed 503 nasopharyngeal swabs collected during 2008–2009 influenza season from ILI patients of the Baganuur district of Ulaanbaatar and Selenge province, Mongolia, and 7000 nasopharyngeal swabs collected in 133 ISSSs during 2009–2010 influenza season.

