Veterinary Integrative Sciences 2023; 21(2): 265 - 271 DOI; 10.12982/VIS.2023.021

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Human rapid influenza diagnostic test: efficacy and significance for using in animal

Duanghathai Saipinta[1], Phongsakorn Chuammitri[2], Satoshi Ohkura[3], Banlang Luangwaranan[4] and Pakpoom Tadee[5,*]

[1]Ph.D.'s Degree Program in Veterinary Science, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand.
[2]Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Research Center of Producing and Development of Products and Innovations for Animal Health and Production, Chiang Mai University
[3]Laboratory of Animal Production Science, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan
[4]Hausen Bernstein Co., Ltd., Bangkok 10140, Thailand
[5]Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand.

Abstract

Rapid influenza diagnostic tests (RIDTs) could considerably assist in clinical management resolving outbreaks of diseases in humans and animals. The RIDT for animals is rarely available and considerably expensive, especially in Thailand. In this study, therefore, the objective of this study was to determine the capacity of a commercial human RIDT "QuickNavi™Flu2", an RIDT for influenza viruses A and B, to detect influenza viruses A antigen including 2 inactivated swine influenza viruses: H1N1 and H3N2, canine parainfluenza virus, and feline calicivirus. In addition, the efficacy of QuickNavi™Flu2 was compared with the real-time polymerase chain reaction assay (real-time rt-PCR). Both of SIV were used as stock solutions, and were serially diluted 6 concentrations before testing. The stock and diluted solutions of both SIV, specimens of canine parainfluenza virus, feline calicivirus and 96 unknown samples were tested by QuickNavi™Flu2 according to the guideline. Then all of samples were repeated testing with real-time rt-PCR assay. Results showed that Quick Navi-Flu could not give positive results for dilutions lower than 1:10 and 1:100, respectively. The sensitivity and specificity values of QuickNavi™Flu2 test in comparison to real-time rt-PCR assay for influenza A virus were 36.1% and 100%, respectively. In conclusion, a human RIDT can detect swine influenza H1N1 and H3N2 in high viral concentration, and appeared to be a useful tool for screening in animals when the viral load is sufficient.

Keywords: Influenza A virus, QuickNavi™Flu2, Rapid influenza diagnostic tests, Real-time rt-PCR, Swine influenza

Corresponding author: Pakpoom Tadee, Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand. Tel: +66-(0)-53-948-02-3, E-mail: td.pakpoom@gmail.com

Article history; *received manuscript: 1 December 2022,*
revised manuscript: 14 Janury 2023,
accepted manuscript: 14 February 2023,
published online: 22 February 2023
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Influenza is one of the most prevalent infectious diseases in the world. The influenza virus can infect humans and many kinds of animals. This virus causes acute respiratory infection and can be transmitted easily from infected individuals to other people, animal to animal, and animal to people (Richard and Fouchier, 2016). This virus can be infected with a broad host range, poultry and mammalian species such as humans, pigs, horses, dogs and cats (Kaplan and Webby, 2013). Swine influenza viruses (SIV) identified in Thailand were H1N1 (29%), H3N2 (12.9%) and H1N2 (3.2%) strain (Takemae et al., 2008; Sreta et al., 2009). Pig that infected with SIV was probably 100% of morbidity rates and have a bad affected on pig's health seriously.

One of the most essential procedures to control epidemic diseases before its transmission into the large population is early diagnosis of disease (Andresen and Kesson, 2010). A variety of different diagnostic methods can be used to detect the presence of influenza viruses in respiratory specimens, including direct antigen detection tests (Chidlow et al., 2010), virus isolation in cell cultures, and detection of influenza-specific RNA by real-time reverse transcriptase (RT)-polymerase chain reaction (PCR) (Kang et al., 2010). While the gold standard for the diagnosis of influenza is virus isolation using chicken embryos or tissue culture method, this protocol needs a standard laboratory and time consumption that might not be suitable for disease control program (Pabbaraju et al., 2009).

A human rapid influenza diagnostic tests (RIDT) is the test kit for the differential diagnosis of Influenza A and B viral strains in fresh nasal or throat swab specimens, providing fast supplemental test results to support early clinical diagnosis of influenza in human. This easy-to-perform test does not require any specific equipment (such as needed for PCR) and can be performed in any setting, thereby offering test results in less than 10 minutes in farms to start disease control program without any further delay. However, the use of human test kit on animal needs to be validated before using as an animal test kits. Therefore, the objectives of this study are to determine the detection level of virus concentrations and the possibility to use human RIDT as the test kits for animals.

MATERIALS AND METHODS

All animal care and sample collection protocol were approved by the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Chiang Mai University (approval no. S3/2565).

- Specimens and Samples preparation

Two of inactivated swine influenza viruses; H1N1 (A/swine/Thailand/CU-S3246N/2012) and H3N2 (A/swine/Thailand/CU-S3673N/2012) were used as positive stock solutions, and using saline, were serially diluted to 1:10, $1:10^2$, $1:10^3$, $1:10^4$ and $1:10^5$. Both of inactivated swine influenza virus samples were provided courtesy of the Faculty of Veterinary Medicine, Chulalongkorn

University, Thailand. The negative specimens were canine parainfluenza virus and feline calicivirus. Ninety-six unknown nasal swab samples were collected form animals (8 dogs, 23 cats, 20 pigs and 45 dairy cattle), using the nasal swab collecting sets, inserted into a transport tube containing viral transport media, kept on ice, transported to the laboratory within 24 hours, and kept at -80 °C until use.

- Detection limit and stability assessment

A total of 138 specimen sample consisted of the stock and diluted solutions of both inactivated swine influenza viruses, specimens of canine parainfluenza virus and feline calicivirus and 96 nasal swab samples in this experiment were tested by commercial human RIDT "QuickNavi™Flu2" according to the guideline. 50 µl of each sample was added to the test kit specimen buffer and pipetted up-down 5 time. Then, the three drop of sample was dropped to the test kit carefully and were allowed to absorb the fluid thoroughly. The test kit was leave to stand at room temperature (15-30 °C) for 5 minutes and confirmed the presence or absence of lines in the result window.

- Real-time rt-PCR for influenza A virus detection.

All of samples which tested by QuickNavi™Flu2 were tested parallelly with real-time rt-PCR. Viral nucleic acids were extracted from a separate aliquot of each sample using NucleoSpin® RNA Virus kit and cDNA synthesis by ReverTra Ace® qPCR RT Master Mix following the manufacturer's instructions. The cDNA amplification reaction system was set at 20 µL, including 5×RT Master Mix 4 at µL, RNA template at 2 µg, and Nuclease-free Water was added to make the final volume 20 µL. The cDNA synthesis cycle was programmed as follows: 37°C for 15 min, 50°C for 5 min, 98°C for 5 min, and finally held at 4°C. The cDNA product of each sample was tested for influenza A virus by real-time rt-PCR. The real-time rt-PCR assays were performed on a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using SensiFAST™ SYBR® Hi-ROX Kit according to the manufacturer's instructions. Primers and probes were taken from a recent publication (Spackman et al.,2002) specifically to amplify a portion of the M gene of influenza A (forward primer: 5'AGATGAGTCTTCTAACCGAGGTCG 3' and reverse primer: 5' TGCAAAAACATCTTCAAGTCTCTG 3'). The positive CT value was 1- 40 and more than 40 was determined as a negative result (Charoenvisal et al., 2013).

- Data analysis.

Considering the real-time rt-PCR as the gold standard for the detection of influenza viruses in respiratory specimens, we determined the diagnostic value of the QuickNavi™Flu2 by calculating its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, and their corresponding 95% confidence intervals (CI) using https://www.medcalc.org/calc/diagnostic_test.php. Comparisons of CT values of the positive and negative test kit result and their detected H1N1 and H3N2 viruses were compared by t-test using Microsoft® Excel version 2019 and deemed significant at a p-value of less than 0.05.

Table 1 Result of the concentration of swine influenza virus detection by QuickNavi™ Flu2 and real-time rt-PCR (+ : positive, w+ : weak positive, vw+ : very weak positive)

	Swine influenza (H1N1)						Swine influenza (H3N2)					
	stock solution	**1:10**	**$1:10^2$**	**$1:10^3$**	**$1:10^4$**	**$1:10^5$**	**stock solution**	**1:10**	**$1:10^2$**	**$1:10^3$**	**$1:10^4$**	**$1:10^5$**
QuickNavi™Flu2	+	w+	-	-	-	-	+	w+	vw+	-	-	-
Realtime rt-PCR	+	+	+	+	+	-	+	+	+	+	+	-

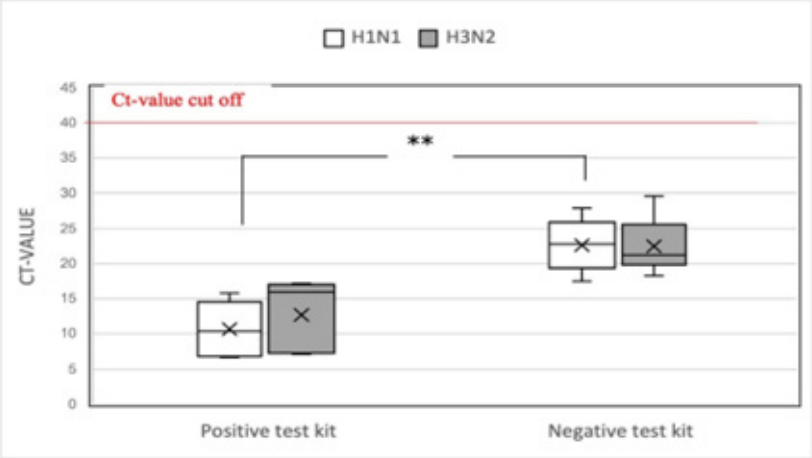

Figure 1 the Ct-values of the positive and negative test kit result and their detected H1N1 and H3N2 viruses (** indicate different CT values at $p < 0.05$).

Table 2 Comparison of diagnosis efficacy of QuickNavi™ Flu2 based on real-time rt-PCR result; n = 138 (n: number, CI: confidence interval)

	Real-time rt-PCR	
	Positive	**Negative**
QuickNavi™ Flu2 $^+$, n	13	0
QuickNavi™ Flu2 $^-$, n	23	102
Sensitivity, % (95% CI)	36.1 (20.8 -53.8)	
Specificity, % (95% CI)	100.0 (96.5 -100.0)	
Positive predictive value, % (95% CI)	100.0 (75.3 - 100.0)	
Negative predictive value, % (95% CI)	81.6 (73.7 - 88.0)	
Accuracy, % (95% CI)	83.3 (76.1 - 89.1)	

RESULTS

In this experiment, the commercial kits QuickNavi™ Flu2 showed the detection limit for inactivated SIV subtype H1N1and H3N2 where the kits could not give positive result for dilutions lower than 1:10 and 1:100, respectively. For the real-time rt-PCR result, both of SIV were detected at the high dilution of 1:10,000 (Table 1). The test kit gave negative result for canine parainfluenza virus and feline calicivirus.

QuickNavi™ Flu2 were reported as positive with CT values of real-time rt-PCR assay as low as 10.68 (range 6.71-15.72, median 10.34) in H1N1

and 12.63 (range 7.24-17.20, median 15.92) in H3N2. Comparisons of CT values of the positive and negative test kit result and their detected H1N1 and H3N2 viruses were significantly different ($p<0.05$), shown in Figure 1.

None of the unknown sample was positive with the QuickNavi™ Flu2 test, but 6 unknown samples were detected with influenza A virus by Realtime rt-PCR. The efficiency of QuickNavi™ Flu2 in comparison with real-time rt-PCR was shown in Table 2. QuickNavi™ Flu2 showed high specificity in the detection of influenza A virus, but relatively low sensitivity.

DISCUSSION

Due to the pandemic of influenza virus emerged in human and animal. The rapid tests for influenza became more widely used and more readily available was produced for the strains of circulating influenza viruses (Peaper and Landry, 2014). The advantages of rapid immunoassays included their ease of use, particularly under field conditions, with results available quickly after commencing the test (Green et al., 2018). In this study, QuickNavi™ Flu2 which is the commercial RIDT for humans detected the positive SIV samples that were derived from infected pigs in Thailand. The RIDT was found to be able to detect swine influenza even after the virus had been inactivated. Although the RIDT ability to detect the virus showed positive results in relatively high concentrations of virus.

Lower Ct values on real-time rt-PCR in our samples was associated with a higher positivity of RIDT tests, suggesting that a higher viral load was associated with a higher positivity of the RIDT antigen test. This finding is similar to a recently published report. There was an inverse relationship between Ct values and viral load and those therefore qualitative results from rt-PCR assays could be converted into quantitative viral load values in clinical samples (Piralla et al., 2013). In addition, the recent reports suggested that the double nasal swabs used in the rt-PCR samples might also have yielded a higher positivity because of a higher viral load due to a greater volume of the specimen collected by two swabs as compared to one swab in the RIDT test (Louie et al., 2010). We collected samples from the nasal cavity which is the standard method for collecting influenza samples. Previous reports have suggested that nasal swab, nasopharyngeal swab, throat swab sampling was not significantly different in viral detection (Decorte et al., 2015)

In this study, the QuickNavi™ Flu2 showed relatively low sensitivity (36.1%) and high specificity (100%) when tested with total 138 samples (SIV sample, negative samples and unknown samples) and compared with the real-time rt-PCR detection of the same specimen. This is similar to study of specific RIDT test kit for the detection of H5, it has been reported in the detection of avian influenza H5N1, and found that the sensitivities of these tests ranged from 36.3% to 51.4%, which was lower than those using genome detection methods (Chua et al., 2007). The study of human RIDT, when tested in humans showing signs of influenza-like illness, found that variable performance of RIDT, with reported sensitivities ranging from 27% - 61%, when compared with real-time rt-PCR testing (Crum-Cianflone et al., 2009; Jernigan et al., 2011). Thus, false negative results occurred more commonly than false positive results. However, previous study of QuickNavi™ Flu that was tested in Japanese human adults with influenza-like symptoms during outbreak of

influenza A virus, The sensitivities and specificities were 86.8% and 98.8% for influenza A, respectively (Drexler et al., 2009).

Negative results of RIDTs do not necessarily mean that the influenza virus is not detected in animals and should still be considered in illness animals (Chauhan et al., 2013). Although the RIDTs were able to detect virus at the highest virus concentrations, some were unable to detect certain viruses at any subsequent dilution. In addition, if the results of RIDT is a negative result in illness animals that suspected of influenza infection. Veterinarian and clinicians can measures to improve detection of influenza, such as, collecting specimens early in the course of illness, ensuring that the appropriate type and highest quality of respiratory specimen was collected, and using the current local prevalence of influenza activity to increase or decrease the suspicion of influenza and to assess the benefit of testing (CDC, 2010).

In conclusion, a human RIDT can detect H1N1 and H3N2 in high viral concentration, and therefore, it cannot be used for influenza diagnosis in mild respiratory signs animals or in non-epidemic conditions. Overall, the results indicated that the QuickNavi™Flu2 was probably not to be used in the diagnostic of influenza for the screening tests in animals.

ACKNOWLEDGEMENTS

We would like to thank the Research and Researchers for Industries Ph.D. program (PHD62I0016), CU-EIDAs (Faculty of Veterinary Science, Chulalongkorn University) and Hausen Bernstein Co., Ltd.

REFERENCES

Andresen, D.N., Kesson, A.M., 2010. High sensitivity of a rapid immunochromatographic test for detection of influenza a virus 2009 H1N1 in nasopharyngeal aspirates from young children. J. Clin. Microbiol. 48(7), 2658-2659.

Centers for Disease Control and Prevention. Rapid diagnostic testing for influenza information for health care professionals Available online: http://www.cdc.gov/flu/professionals/ diagnosis/rapidclin.htm. (Accessed on March 1, 2010).

Charoenvisal, N., Keawcharoen, J., Sreta, D., Tantawet, S., Jittimanee, S., Arunorat, J., Amonsin. A., Thanawongnuwech, R., 2013. Experimental infection with a Thai reassortant swine influenza virus of pandemic H1N1 origin induced disease. Virol. J. 10(1), 88.

Chauhan, N., Narang, J., Pundir, S., Singh, S., Pundir, C.S., 2013. Laboratory diagnosis of swine flu: a review. Artif. Cells. Nanomed. Biotechnol. 41(3), 189–195.

Chidlow, G., Harnett, G., Williams, S., Levy, A., Speers, D., Smith, D.W., 2010. Duplex real-time reverse transcriptase PCR assays for rapid detection and identification of pandemic (H1N1) 2009 and seasonal influenza A/H1, A/H3, and B viruses. J. Clin. Microbiol. 48(3), 862-866.

Chua, T-H., Ellis, T.M., Wong, C.W., Guan, Y., Ge, S.X., Peng, G., Lamichhane, C., Maliadis, C., Tan, S., Selleck, P., 2007. Performance evaluation of five detection tests for avian influenza antigen with various avian samples. Avian. Dis. 51(1), 96–105.

Crum-Cianflone, N.F., Blair, P.J., Faix, D., Arnold, J., Echols, S., Sherman, S.S., Tueller, J.E., Warkentien, T., Sanguineti, G., Bavaro, M., 2009. Clinical and epidemiologic characteristics of an outbreak of novel H1N1 (swine origin) influenza A virus among United States military beneficiaries. Clin. Infect. Dis. 49(12), 1801–1810.

Decorte, I., Steensels, M., Lambrecht, B., Cay, A.B., De Regge, N., 2015. Detection and isolation of swine influenza a virus in spiked oral fluid and samples from individually housed, experimentally infected pigs: Potential role of porcine oral fluid in active influenza a virus surveillance in swine. PLoS One. 10(10), e0139586.

Drexler, J.F., Helmer, A., Kirberg, H., Reber, U., Panning, M., Müller, M., Höfling, K., Matz, B., Drosten, C., Eis-Hübinger, A.M., 2009. Poor clinical sensitivity of rapid antigen test for influenza a pandemic (H1N1) 2009 virus. Emerg. Infect. Dis. 15(10), 1662-1664.

Green, D.A., StGeorge, K., 2018. Rapid antigen tests for influenza: Rationale and significance of the FDA reclassification. J. Clin. Microbiol. 56(10), e00711-18.

Jernigan, D.B., Lindstrom, S.L., Johnson, J.R., Miller, J.D., Hoelscher, M., Humes, R., Shively, R., Brammer, L., Burke, S.A., Villanueva, J.M., Balish, A., Uyeki, T., Mustaquim, D., Bishop, A., Handsfield, J.H., Astles, R., Xu, X., Klimov, A.I., Cox, N.J., Shaw, M.W., 2011. Detecting 2009 pandemic influenza a (H1N1) virus infection: availability of diagnostic testing led to rapid pandemic response. Clin. Infect. Dis. 52(Suppl 1), S36-43.

Kang, X., Jiang, T., Li, Y., Lin, F., Liu, H., Chang, G., Zhu, Q., Qin, E., Qin, C., Yang, Y., 2010. A duplex real-time RT-PCR assay for detecting H5N1 avian influenza virus and pandemic H1N1 influenza virus. Virol. J. 7(1), 113.

Kaplan, B.S., Webby, R.J., 2013. The avian and mammalian host range of highly pathogenic avian H5N1 influenza. Virus. Res. 178(1), 3–11.

Louie, J.K., Guevara, H., Boston, E., Dahlke, M., Nevarez, M., Kong, T., Schechter, R., Glaser, C.A., Schnurr, D.P., 2010. Rapid influenza antigen test for diagnosis of pandemic (H1N1) 2009. Emerg. Infect. Dis. 16(5), 824–826.

Pabbaraju, K., Wong, S., Wong, A.A., Appleyard, G.D., Chui, L., Pang, X.L., Yanow, S.K., Fonseca, K., Lee, B.E., Fox, J.D., Preiksaitis, J.K., 2009. Design and validation of real-time reverse transcription-PCR assays for detection of pandemic (H1N1) 2009 virus. J. Clin. Microbiol. 47(11), 3454-3460.

Peaper, D.R., Landry, M.L., 2014. Rapid diagnosis of influenza: state of the art. Clin. Lab. Med. 34(2), 365–385.

Piralla, A., Daleno, C., Pariani, E., Conaldi, P., Esposito, S., Zanetti, A., Baldanti, F., 2013. Virtual quantification of influenza A virus load by real-time RT-PCR. J. Clin. Virol. 56(1), 65–68.

Richard, M., Fouchier, R.A., 2016. Influenza A virus transmission via respiratory aerosols or droplets as it relates to pandemic potential. FEMS Microbiol. Rev. 40(1), 68–85.

Spackman, E., Senne, D.A., Myers, T.J., Bulaga, L.L., Garber, L.P., Perdue, M.L., Lohman, K., Daum, L.T., Suarez, D.L., 2002. Development of a real-time reverse transcriptase pcr assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. J. Clin. Microbiol. 40(9), 3256-3260.

Sreta, D., Kedkovid, R., Tuamsang, S., Kitikoon, P., Thanawongnuwech, R., 2009. Pathogenesis of swine influenza virus (Thai isolates) in weanling pigs: an experimental trial. Virol. J. 6(1), 34.

Takemae, N., Parchariyanon, S., Damrongwatanapokin, S., Uchida, Y., Ruttanapumma, R., Watanabe, C., Yamaguchi, S., Saito, T., 2008. Genetic diversity of swine influenza viruses isolated from pigs during 2000 to 2005 in Thailand. Influenza. Other. Respir. Viruses. 2(5), 181-189.

How to cite this article;

Duanghathai Saipinta, Phongsakorn Chuammitri, Satoshi Ohkura, Banlang Luangwaranan and Pakpoom Tadee. Human rapid influenza diagnostic test: efficacy and significance for using in animal. Veterinary Integrative Sciences. 2023; 21(2): 265 - 271.