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Detection sensitivity of influenza rapid diagnostic tests

Yuko Sakai-Tagawa¹, Makoto Ozawa^{2,3}, Shinya Yamada¹, Yuko Uchida⁴, Takehiko Saito⁴, Kazuo Takahashi⁵, Norio Sugaya⁶, Masato Tashiro⁷, and Yoshihiro Kawaoka^{1,8,9,10,11,*}

¹Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639, Japan

²Laboratory of Animal Hygiene, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima 890-0065, Japan

³Transboundary Animal Disease Center, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Kagoshima 890-0065, Japan

⁴Influenza and Prion Research Center, National Institute of Animal Health, Kannondai 3-1-5, Tsukuba, Ibaraki 305-0856, Japan

⁵Department of Infectious Diseases, Osaka Prefectural Institute of Public Health, Osaka, Osaka 537-0025, Japan

⁶Department of Pediatrics, Keiyu Hospital, Yokohama 220-0012, Japan

⁷Influenza Virus Research Center, National Institute of Infectious Diseases, Musashimurayama, Tokyo 208-0011, Japan

⁸ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, Saitama 332-0012, Japan

⁹Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639, Japan

¹⁰Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 575 Science Drive, Madison, WI 53711, USA

¹¹Laboratory of Bioresponses Regulation, Department of Biological Responses, Institute for Virus Research, Kyoto University, Kyoto, Kyoto 606-8507, Japan

Abstract

We compared the sensitivity of influenza rapid diagnostic tests (IRDTs) currently available in Japan for various influenza virus strains, including human H7N9 and H5N1 isolates. We found that all of the IRDTs examined detected these viruses, but their detection sensitivities differed.

*Corresponding author. Mailing address: Yoshihiro Kawaoka Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Phone: 03-5449-5310 Fax: 03-5449-5408 kawaoka@ims.u-tokyo.ac.jp.

Disclosure

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Keywords

Influenza; rapid diagnostic tests; detection sensitivity; avian H7N9 and H5N1 viruses

Influenza virus infections cause acute respiratory diseases and easily transmit among humans. Because of the fast clinical course and the potential to spread quickly, rapid and easy diagnosis is essential to minimize the damage caused by influenza virus infection. A large number of influenza rapid diagnostic tests (IRDTs) have been developed and are widely used in clinical practice to diagnose the so-called “seasonal influenza” that is caused by H1N1 and H3N2 influenza A viruses and influenza B viruses. In particular, IRDTs are important for antiviral treatment of influenza patients, because neuraminidase (NA) inhibitors, including oseltamivir (1) and zanamivir (2), are most effective if given within two days of the onset of symptoms (3). In fact, more than 20 different IRDTs are available in Japan, where anti-influenza drugs are prescribed routinely.

Besides seasonal influenza viruses, influenza viruses from other species have sporadically transmitted to humans. For example, human infections with the recently emerged avian H7N9 influenza viruses in March 2013 in China (4-6) now pose a new threat to public health. As of 29 January 2014, 238 confirmed cases with 56 deaths have been reported mainly in China. Although the first wave ended in late spring, sporadic human cases have continued to be reported and we are now faced with a second wave (http://www.who.int/csr/don/2013_10_24a/en/index.html). In addition, highly pathogenic avian influenza H5N1 viruses are endemic in poultry populations in Eastern Asia and Egypt, and continue to infect humans (http://www.who.int/entity/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_20December13.pdf). Because vaccines against emerging viruses cannot be prepared in a timely manner, the first option to control infection with such viruses is antiviral drug treatment. Therefore, IRDTs play a vital role in the initial diagnosis of patients exhibiting influenza-like symptoms.

Previously, we (7) and others (8, 9) demonstrated that the H7N9 viruses are sensitive to currently licensed NA inhibitors, such as oseltamivir, zanamivir, laninamivir (10), and peramivir (11), and to an inhibitor of the viral RNA polymerase, favipiravir [(12), also known as T-705]. Although some H5N1 isolates show reduced sensitivity to oseltamivir (13-18), both laninamivir and favipiravir have been shown to be effective against oseltamivir-resistant viruses (19, 1). Because these antiviral drugs should be administered as early as possible after the onset of symptoms to be efficacious, rapid and easy diagnosis of influenza infection is essential to help minimize the individual and social damage caused by H7N9 and/or H5N1 influenza virus infection. IRDTs, thus, should be useful to control sporadic human infections with these viruses.

Because most IRDTs are optimized for the detection of seasonal influenza viruses, their sensitivity for other influenza viruses should be individually tested. Previously, we compared the detection sensitivity of 20 IRDTs available in 2009 in Japan for various virus strains, including the earliest isolates of pandemic (H1N1) 2009 viruses and highly pathogenic avian influenza H5N1 viruses (20). After the 2009 influenza pandemic, IRDTs available in Japan were updated or improved, and new ones were developed. Here, we

evaluated the sensitivity of the IRDTs currently available in Japan for various influenza virus strains, including recent human isolates of the H7N9 and H5N1 subtypes.

Twenty one IRDTs commercially available in Japan in April through September 2013 were examined in this study (Table 1). All of the IRDTs are designed to detect influenza virus by using an NP-specific monoclonal antibody against influenza viral nucleoprotein (NP), which is a major virion component that is highly conserved among influenza A and B viruses. The IRDTs could be divided into two format types: well or test strip format. The detection mechanism for all of the IRDTs is based on the immunochromatographic method, with the exception of that for Immunotrap InfluenzaA•B, whose reaction is mediated by magnetic energy. The main factors affecting the detection sensitivity of the IRDTs for various influenza viruses are affinity, specificity, and cross-reactivity of the NP-specific monoclonal antibody; however, composition of the lysis buffer, the dilution rate of the specimens, and the method used to visualize the test results may also affect the detection sensitivity. Most test results are available in 1–15 min.

Influenza viruses of various subtypes (Table 2) were propagated in Madin-Darby canine kidney (MDCK) cells or in embryonated chicken eggs and stored at -80°C until use. The virus titers were determined by using 50% tissue culture infectious dose (TCID₅₀) assays in MDCK cells. Ten-fold serial dilutions of viruses (10¹ to 10⁶ TCID₅₀/100 µl) with Eagle's minimal essential medium containing 0.3% bovine serum albumin were applied to each IRDT as specimens according to the manufacturers' instructions. The minimum viral titers required for the positive reaction in each IRDT were determined twice independently. The average titers of the two experiments were then considered as the detection limit for each IRDT.

The detection sensitivity for seasonal influenza viruses of all of the IRDTs examined were relatively higher than those for non-seasonal isolates (Table 3). In particular, most of the IRDTs gave a positive result with 10³–10⁴ TCID₅₀ of the currently circulating H1N1 viruses that originated from the pandemic (H1N1) 2009 viruses. The IRDTs were less sensitive for the detection of the seasonal H3N2 viruses.

Most of the IRDTs were even less sensitive for type B viruses. By phylogenetically analyzing the NP genes of B/Yokohama/UT-K1A/11 (Type B-1), B/Yokohama/UT-K31/12 (Type B-2), and representative isolates (21, 22), we found that the NP genes of recent influenza B viruses are divided into two lineages (Figure 1) that are unrelated to the HA gene-based Victoria and Yamagata lineages (23). The NP genes of Type B-1 and -2 viruses belong to each of these lineages, indicating that these two viruses represent currently circulating strains.

Intriguingly, the detection sensitivities of the IRDTs for five H7 isolates, including two early human isolates of the recent H7N9 outbreak in China, were comparable to those for the seasonal H3N2 viruses (Table 4). Likewise, the detection sensitivities for the H5N1 isolates tested were largely comparable to those for the seasonal H3N2 viruses with a few exceptions (Table 5). Overall, the detection sensitivities of the IRDTs did not correlate with the host species; for example, the detection sensitivities of most of the IRDTs for A/chicken/East

Kalimantan/UT581/10 (H5N1; H5N1-8) were comparable to those for A/Osaka/UT-A01/13 (H1N1; H1N1-2), which was the most reactive to the IRDTs among the seasonal viruses tested.

In our previous study (20), some of the IRDTs available at that time could not detect some H5N1 viruses, such as A/Vietnam/UT3040/04 (H5N1), even at 10^6 TCID₅₀. By contrast, all of the IRDTs examined in this study detected A/Vietnam/UT3040/04 (H5N1; H5N1-9) (Table 5), as well as all of the other viruses tested. These results suggest that the IRDTs examined in this study are more reliable than ever for the detection of non-seasonal influenza viruses, including the newly emerged H7N9 and the recently circulating H5N1 viruses; they are thus useful to detect influenza infection even in patients infected with H7N9 or H5N1 viruses.

In summary, we evaluated the detection sensitivity of 21 IRDTs for 20 influenza virus strains, including recent human isolates of the H7N9 and H5N1 subtypes. We found that all of the IRDTs examined in this study detected all of the viruses tested although the detection sensitivities differed among the IRDTs and type B viruses were less reactive. Our results suggest that contemporary IRDTs are useful to control sporadic human infections with H7N9 or H5N1 viruses, as well as seasonal influenza viruses.

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List of abbreviations

IRDT	influenza rapid diagnostic test
NA	neuraminidase
NP	nucleoprotein
MDCK	Madin-Darby canine kidney

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Figure 1. Phylogenetic analysis of the NP gene of type B viruses

The nucleotide sequences of the NP genes from the type B viruses used in this study (i.e., B/Yokohama/UT-K1A/11 and B/Yokohama/UT-K31/12) and from representative type B viruses available in GenBank were phylogenetically analyzed by using the maximum likelihood method in the MEGA 6 software (24) with a bootstrapping set of 100 replicates. Open and closed circles represent the Victoria and Yamagata lineages, respectively. The viruses that were isolated since 1988, and are still circulating are divided into two lineages (I and II) based on their NP gene sequences. The scale bar indicates the number of nucleotide substitutions per site.

Table 1

Influenza rapid diagnostic tests examined.

IRDT	Manufacturer	Time (min) [†]	Dilution rate (%) [‡]	Format [§]
ESPLINE® Influenza A & B-N	Fujirebio Inc.	15	6.7	Well
ImmunoAce®Flu	Tauns Laboratories, Inc.	3-8	12.5	Well
BD Veritor™ system Flu	Beckton, Dickinson and Co.	5-10	13.3	Well
Quick Chaser® Flu A,B(Type H)	Mizuho Medy Co., Ltd.	5-10	16.7	Well
RapidTest® FLU NEO	Sekisui Medical Co., Ltd.	15	19.2	Well
Clearview® Exact Influenza A&B	Alere Medical	8	66.7	Test strip
Prorast Flu	Mitsubishi Chemical Medience Co.	10	10.0	Well
BD Flu Examan™	Beckton, Dickinson and Co.	15	11.4	Well
RapidTest® color FLU stick	Sekisui Medical Co., Ltd.	2-10	75.0	Test strip
QuickVue® Rapid SP Influenza test	DS Pharma Biomedical Co., Ltd.	10	75.0	Test strip
QuickNav™ Flu	Denka Seiken Co., Ltd.	3-8	10.0	Well
QuickNav™ Flu+RSV	Denka Seiken Co., Ltd.	3-8	10.0	Well
Statmark™ FLU Stick-N	Nichirei Biosciences Inc.	1-10	25.0	Test strip
Primecheck® Flu · RSV	Alfresa Pharma Co.	5-10	15.0	Well
CHECK Flu A-B	Alfresa Pharma Co.	3-8	15.0	Well
ClearLine® InfluenzaA/B/H1N1/2009	Meiji Seika Pharma Co	10-15	75.0	Test strip
POCTEM® S Influenza	Symex Co.	8	22.2	Test strip
Brighpoc®Flu	Nichirei Biosciences Inc.	1-10	13.8	Well
Immunofine™(FLU)	Nichirei Biosciences Inc.	1-10	13.8	Well
Caplia® Flu A+B	Tauns Laboratories, Inc.	3-8	12.5	Well
Immunotrap InfluenzaA-B	Wako Pure Chemical Industries, Ltd.	1	10.0	Well

[†]The time required to obtain the test results is based on the individual manufacturer's instructions.

[‡]For all IRDTs examined, the test specimen (A) had to be suspended in a diluent (B). Subsequently, all or part of the diluent (C) was subjected to the assay. Dilution rates were calculated using the following formula: $\text{volume C} / (\text{volume A} + \text{volume B}) \times 100$.

[§]Based on their format, all IRDTs examined were divided into one of two types: well format, the diluted specimen is dropped onto the wells and the reaction occurs inside a covered plastic body; or test strip format, the test strip is dipped into the diluted specimen and the reaction occurs on the strip.

Table 2

Influenza virus strains tested.

Category [†]	Virus strain name	Host	Subtype or type [‡]	Abbreviation [§]
Seasonal viruses	A/Yokohama/UTK2A/11 (H1N1)	Human	H1N1	H1N1-1
	A/Osaka/UT-A01/13 (H1N1)	Human		H1N1-2
	A/Yokohama/UT-K4A/11 (H3N2)	Human	H3N2	H3N2-1
	A/Tokyo/UT-IMS6-1/13 (H3N2)	Human		H3N2-2
	B/Yokohama/UT-K1A/11	Human	Type B	Type B-1
	B/Yokohama/UT-K31/12	Human		Type B-2
H7 viruses	A/Duck/Gunma/466/11 (H7N9)	Duck	H7N9	H7N9-1
	A/Anhui/1/13 (H7N9)	Human		H7N9-2
	A/Shanghai/1/13 (H7N9)	Human		H7N9-3
	A/Duck/Hong Kong/301/78 (H7N2)	Duck	H7N2	H7N2
	A/Seal/Massachusetts/1/80 (H7N7)	Seal	H7N7	H7N7
Highly pathogenic H5N1 viruses	A/Vietnam/UT31604/09 (H5N1)	Human	2.3.4.2	H5N1-1
	A/Duck/Vietnam/TY165/10 (H5N1)	Duck	2.3.2	H5N1-2
	A/Chicken/Egypt/119S-NLQP/11 (H5N1)	Chicken	2.2.1	H5N1-3
	A/Chicken/Indonesia/NC/09 (H5N1)	Chicken	2.1.3.2	H5N1-4
	A/Vietnam/UT362851/10 (H5N1)	Human	H5N1 2.3.4.1	H5N1-5
	A/Duck/Vietnam/TY151/11 (H5N1)	Duck	2.3.2	H5N1-6
	A/Chicken/Egypt/0977-NLQP/09 (H5N1)	Chicken	2.2.1.1	H5N1-7
	A/Chicken/East Kalimantan/UT581/10 (H5N1)	Chicken	2.1.3.1	H5N1-8
	A/Vietnam/UT3040/04 (H5N1)	Human	1	H5N1-9

[†]Viruses tested are categorized into three groups based on their properties.

[‡]For H5N1 viruses, the clade of each strain is also shown.

[§]Abbreviations used in Tables 3–5 are shown.

Table 3

Detection sensitivity for seasonal influenza viruses[†].

IRDT	Minimum viral titer (log ₁₀ TCID ₅₀ /100 µl) for a positive reaction with:					
	H1N1-1	H1N1-2	H3N2-1	H3N2-2	TypeB-1	Type B-2
ESPLINE® Influenza A & B-N	3	3	3	4	4.5	5
ImmunoAce®Flu	3	3	4	4	4	4.5
BD Veritor™ system Flu	3	3	4	4	4	5
Quick Chaser® Flu A,B(Type H)	4	3	4	5	4	5
RapidTesta® FLU NEO	4	3.5	4	4.5	4	4.5
Clearview® Exact Influenza A&B	4	3	4	4	5	6
Prorast Flu	4	3	4	4	5	6
BD Flu Examan™	4	3	4.5	5	5	5
RapidTesta® color FLU stick	4	3.5	4	4	5	5
QuickVue® Rapid SP Infl u test	4	3	4	4	5	5.5
QuickNavit™ Flu	4	3	4	5	5	5.5
QuickNavit™ Flu+RSV	4	4	4.5	5	5	6
Statmark™ FLU Stick-N	4	4	4	5	5	5
Primecheck® Flu · RSV	4	4	4.5	5	4.5	5
CHECK Flu A·B	4	4	4.5	5	5	5
ClearLine®InfluenzaA/B/H1N12009	4	4	5	5.5	4	5
POCTEM® S Influenza	4	4	4.5	5	5	5.5
Brighpoc®Flu	4	4	4	5	5	6
Immunofine™FLU)	4	4	4	5	5	6
Capilia® Flu A+B	4	4	4.5	5	5	5.5
Immunotrap InfluenzaA·B	5	4	5	5	5	6

[†]Ten-fold serial dilutions of the indicated viruses (10^1 to 10^6 TCID₅₀/100 μ l) were subjected to each IRDT as specimens according to the manufacturers' instructions. The minimum viral titers required for a positive reaction were determined in two independent experiments. The average titers are shown.

Table 4

Detection sensitivity for H7 influenza viruses[†].

IRDT	Minimum viral titer (log ₁₀ TCID ₅₀ /100 µl) for a positive reaction with:					
	H7N9-1	H7N9-2	H7N9-3	H7N2	H7N7	
ESPLINE® Influenza A & B-N	4	5	4	3	3	
ImmunoAce®Flu	4	5	4	3	3.5	
BD Veritor™ system Flu	4	5	4.5	3.5	4	
Quick Chaser® Flu A,B(Type H)	4	5	5	4	4	
RapidTest® FLU NEO	4.5	5.5	4	3.5	4	
Clearview® Exact Influenza A&B	4	5	5	4	4	
Prorast Flu	4	5	5	3.5	4	
BD Flu Examan™	4.5	5	5	3.5	4	
RapidTest® color FLU stick	4.5	5.5	5	3.5	4	
QuickVue® Rapid SP Influenza	5	5	5	4	4	
QuickNavit™ Flu	5	5.5	5	4	4	
QuickNavit™ Flu+RSV	4.5	5	5	3.5	4	
Statmark™ FLU Stick-N	5	5	5	4	4	
Primecheck® Flu · RSV	5	5	5	4	4	
CHECK Flu A+B	4.5	5	5	4	4	
ClearLine® Influenza A/B/H1N12009	5	5.5	4.5	4	4	
POCTEM® S Influenza	5	6	4	4	4	
Brighipoc®Flu	5	6	5	4	4	
Immunofine™FLU)	5	6	5	4	4	
Caplia® Flu A+B	5	6	5	4	4.5	
Immunotrap Influenza A/B	6	6	6	4	5	

[†]Ten-fold serial dilutions of the indicated viruses (10¹ to 10⁶ TCID₅₀/100 µl) were subjected to each IRDT as specimens according to the manufacturers' instructions. The minimum viral titers required for a positive reaction were determined in two independent experiments. The average titers are shown.

Table 5

Detection sensitivity for highly pathogenic H5N1 influenza viruses[†].

IRDT	Minimum viral titer (log ₁₀ TCID ₅₀ /100 µl) for a positive reaction with:								
	H5N1-1	H5N1-2	H5N1-3	H5N1-4	H5N1-5	H5N1-6	H5N1-7	H5N1-8	H5N1-9
ESPLINE® Influenza A & B-N	4	4	4	4	4	5	5	3	4
ImmunoAce®Flu	4.5	3.5	4	4	4	5	5	3	4
BD Veritor™ system Flu	4.5	3.5	4	4	4	5	5	2.5	4
Quick Chaser® Flu A,B(Type H)	4.5	4	4	4	4	5	5	3.5	4.5
RapidTest® FLU NEO	5	4	4.5	4	4.5	5	5.5	3.5	5
Clearview® Exact Influenza A&B	4.5	4	4	4	4.5	5	5	3.5	5
Prorast Flu	5	4	4	4.5	4.5	5	5	3.5	5
BD Flu Examan™	5	4	4.5	4.5	4	5	5	3	5
RapidTest® color FLU stick	5	4.5	4.5	4.5	4.5	5	5	3.5	5
QuickVue® Rapid SP Infl u test	5	4	5	4.5	4.5	5	5	3	5
QuickNavi™ Flu	5	4	5	4.5	4.5	5	5	4	5
QuickNavi™ Flu+RSV	5	4	5	5	5	5	5.5	3.5	5
Statmark™ FLU Stick-N	5	4	5	4	5	5.5	6	4	5
Primecheck® Flu · RSV	5	4.5	5	4.5	5	5.5	6	3.5	5
CHECK Flu A-B	5	4.5	5	5	5	5.5	6	3.5	4.5
ClearLine® InfluenzaA/B/H1N12009	5	5	5	5	5	5.5	5.5	3.5	5
POCTEM® S Influenza	5	5	5	5	5	5.5	5.5	4	5
Brightpoc®Flu	5	5	5	4.5	5	5.5	6	3.5	5
Immunofine™FLU)	5	5	5	4.5	5	5.5	6	3.5	5
Capilia® Flu A+B	5	4.5	5	5	5	5.5	6	3.5	5
Immunotrap InfluenzaA-B	6	5	6	5	6	6	6	5	6

[†]Ten-fold serial dilutions of the indicated viruses (10¹ to 10⁶ TCID₅₀/100 µl) were subjected to each IRDT according to the manufacturers' instructions. The minimum viral titers required for a positive reaction were determined in two independent experiments. The average titers are shown.