JCM Accepts, published online ahead of print on 16 June 2010
J. Clin. Microbiol. doi:10.1128/JCM.00439-10






Revised (changes highlighted in yellow)

2nd revised (changes highlighted in blue)

# Sensitivity of influenza rapid diagnostic tests to H5N1 and 2009 pandemic H1N1 viruses

Short Title: Sensitivity of influenza rapid diagnostic tests

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**Abstract**

Simple and rapid diagnosis of influenza is useful to treatment decision-making in the clinical setting. Although many influenza rapid diagnostic tests (IRDTs) are available for the detection of seasonal influenza virus infections, their sensitivity for other viruses, such as H5N1 viruses and the recently emerged swine-origin pandemic (H1N1) 2009 virus, remains largely unknown. Here, we examined the sensitivity of 20 IRDTs to various influenza virus strains, including H5N1 and 2009 pandemic H1N1 viruses. Our results indicate that the detection sensitivity to swine-origin H1N1 viruses varies widely among IRDTs, with some tests lacking sufficient sensitivity to detect the early stages of infection when the virus load is low.

## Introduction

Influenza is one of the primary infectious diseases affecting public health. H1N1 and H3N2 subtypes of human influenza A and type B viruses cause seasonal influenza with high morbidity and mortality, especially in pediatric, geriatric, and immunocompromised patients (25). In addition to the clinical aspects of these infections, influenza epidemics also have a significant impact on our social economy (14). Further, viruses possessing hemagglutinin (HA) and neuraminidase (NA) to which humans are immunologically naïve have the potential to cause global outbreaks or "pandemics".

Rapid diagnosis of influenza during the early stage of infection allows physicians the opportunity to limit the infection and its sequelae by administering the appropriate antiviral drugs to the patient. NA inhibitors (i.e., oseltamivir and zanamivir), which are widely used to treat influenza, must be administered 36-48 h after the onset of symptoms for maximal therapeutic efficacy (18). It is, however, difficult to distinguish influenza from other acute respiratory disorders based purely on clinical signs and symptoms. The availability of a diagnostic test with high sensitivity that can accommodate the large volume of clinical specimens generated during influenza epidemics and pandemics, and that is simple and quick is the holy grail of influenza diagnostics.

Recently, many rapid tests have been made available to diagnose seasonal influenza in clinical practice. These influenza rapid diagnostic tests (IRDTs) may also help detect sporadic human infections with other influenza viruses (e.g., avian H5N1 viruses), which have the potential to cause a pandemic. In fact, the first case of swine-origin pandemic (H1N1) 2009 virus infection in California was diagnosed as influenza A virus infection by use of an IRDT (3). Although the sensitivity of some IRDTs has been

experimentally evaluated (6, 10, 13, 22, 23), studies on the sensitivity of these tests for non-human influenza viruses are limited (24, 27). Further, although the detection sensitivity of some IRDTs to pandemic (H1N1) 2009 viruses has been reported (2, 4, 5, 7, 8, 11, 12, 15, 26), no one has conducted an extensive side-by-side comparison of IRDT sensitivity with multiple isolates and clinical specimens. Here, we compared the sensitivity of 20 IRDTs to detect seasonal H1N1, H3N2, and type B, human and avian H5N1, other subtypes of avian, and pandemic (H1N1) 2009 viruses. Our findings emphasize the importance of selecting the right IRDT for rapid diagnosis of non-seasonal influenza viruses, since the sensitivity of the IRDTs we tested varied by as much as 100-fold.

## Materials and Methods

**Diagnostic tests.** The influenza rapid diagnostic tests (IRDTs) listed in Table 1 were evaluated. All of the IRDTs can differentiate between influenza A and B viruses. We followed the procedures described in the manufacturers' instructions. Test specimens were $10^1$ to $10^6$ 50% tissue culture infectious dose ($TCID_{50}$) of viruses in 100 µl aliquots of culture supernatants or allantoic fluid or undiluted or diluted nasal swab suspensions derived from pandemic (H1N1) 2009 influenza patients (see below for details). All specimens were tested in a single experiment.

**Viruses.** The influenza viruses used are listed in Table 2. Viruses were propagated in Madin-Darby canine kidney (MDCK) cells or in embryonated chicken eggs to make stock viruses. The stock viruses were titrated in MDCK cells, diluted with Eagle's minimal essential medium containing 0.3% bovine serum albumin, and subjected to IRDTs.

**Clinical specimens.** Nasal swabs, collected from influenza patients who presented at Keiyu Hospital (Yokohama, Japan) and tested positive for influenza using an influenza rapid diagnosis kit during the 2009-2010 influenza season (Table 3), were diluted with Eagle's minimal essential medium containing 0.3% bovine serum albumin and subjected to IRDTs. The virus titer for each original specimen was determined in MDCK cells. These viruses were identified as pandemic (H1N1) 2009 influenza viruses by RT-PCR of the NA gene.

## Results and Discussion

**Comparison of influenza rapid diagnostic tests (IRDTs).** We examined 20 IRDTs that are currently commercially available in Japan (Table 1). There were variations among these IRDTs in shape, method of specimen collection, detection mechanism, time required to obtain results, storage temperature, and shelf life. The fundamental detection principle, however, was identical: viral protein contained in the test specimen was detected based on an antigen-antibody reaction with monoclonal antibody(s) specific for nucleoprotein (NP), which is one of the most abundant proteins in influenza virions and shares relatively high homology in both type A and type B viruses (20). Therefore, the sensitivity of the IRDTs was dependent on the level of cross-reactivity of the NP-specific antibody(s) used with the viruses. The dilution rates of the collected specimen (see footnote to Table 1) with the diluents supplied with each IRDT may also have affected the detection sensitivity.

**Detection sensitivity to seasonal virus strains.** First, the detection sensitivity to nine seasonal virus strains isolated from humans between 2005 and 2009 was examined (Table 2). Most of the IRDTs detected $10^3$-$10^4$ $TCID_{50}$ of the seasonal H1N1 virus strains (Table 4). These results indicate that the IRDTs tested are sensitive enough to diagnose seasonal H1N1 virus infection, since the virus titer in nasopharyngeal wash of influenza patients reaches $10^3$-$10^7$ $TCID_{50}$/ml during the first 24-72 h of illness (1). Relatively higher virus titers ($10^4$-$10^6$ $TCID_{50}$) were required to obtain a positive reaction with H3N2 virus strains, with the exception of A/Kawasaki/UT-K20/08 (H3N2) virus, which was the most recently isolated H3N2 strain tested and was efficiently detected at a

sensitivity similar to that of the seasonal H1N1 strains (Table 4). For type B viruses, $10^4$-$10^5$ $TCID_{50}$ of an isolate from 2005 and $10^5$-$10^6$ $TCID_{50}$ of an isolate from 2008 were required to achieve a positive reaction in all of the IRDTs with the exception of ESPLINE® Influenza A & B-N and Clearview Exact Influenza A&B ($10^3$ $TCID_{50}$ was sufficient for the positive reaction), which exhibited marked sensitivity to all of the seasonal virus strains tested (Table 4). These results suggest that the detection sensitivity of some IRDTs to H3N2 and type B viruses is less than that to H1N1 viruses and that these IRDTs may produce a false-negative for some seasonal influenza viruses.

**Detection sensitivity to H5N1 virus strains.** Since their emergence in Hong Kong in 1997, highly pathogenic avian influenza H5N1 viruses have continued to circulate in birds and occasionally transmit to humans (http://www.who.int/csr/disease/avian_influenza/en/index.html). Early detection of H5N1 infection is crucial for effective therapy with antiviral drugs, such as NA inhibitors, and for quarantine procedures to avoid the possible spread of infection. We, therefore, examined the detection sensitivity of these IRDTs to six human (Table 5) and ten avian (Table 6) H5N1 virus strains belonging to various clades. Unlike the seasonal virus strains, the detection sensitivity varied among IRDTs and among H5N1 virus strains tested. Interestingly, there appeared to be a trend of lower sensitivity of the tests against the more recent isolates, with some exceptions. The difference in clade or host did not account for this temporal tendency (compare the results of virus strains categorized as clade 1 in Tables 5 and 6). The differences in reactivity of these viruses to the IRDTs likely arose from differences in the NP amino acid sequence: while A/whooper

swan/Akita/1/08 (H5N1), A/chicken/Kyoto/3/04 (H5N1), A/Hong Kong/213/03 (H5N1), and A/Indonesia/UT3006/05 (H5N1) viruses share 99.8%-100% amino acid sequence homology in NP with A/Vietnam/UT3040/04 (H5N1) virus, A/Hong Kong/483/97 (H5N1) virus shares only 97.8% with this virus strain. These results suggest that while most IRDTs examined in this study could detect H5N1 viruses, their sensitivity may not be high enough to detect H5N1 influenza viruses during the early stage of an infection.

**Detection sensitivity to other subtypes of avian viruses.** During the last decade, sporadic infection of humans with avian viruses other than H5N1 subtype [e.g., H5N2 (19), H7N7 (9, 16), and H9N2 (21)] have been reported. It is important, therefore, that IRDTs be able to detect human infection with viruses of such unusual subtypes. However, when we examined the detection sensitivity of our IRDTs to four avian isolates of non-H5N1 subtypes, we found that, regardless of the IRDTs tested, all four avian viruses required relatively higher virus titers (> $10^4$ $TCID_{50}$ with one exception) for a positive reaction (Table 7). This finding suggests that these IRDTs are not applicable for several subtypes of avian viruses, displaying relatively low sensitivity.

**Detection sensitivity to pandemic (H1N1) 2009 viruses.** The recent emergence of a swine-origin H1N1 virus and its global spread highlight the need for a simple and rapid diagnostic test for patients thought to be infected with this new pandemic virus called “pandemic (H1N1) 2009 virus”. To evaluate the detection sensitivity of our IRDTs to pandemic (H1N1) 2009 viruses, we used three isolates. As shown in Table 8, at least $10^4$ $TCID_{50}$ of virus was required for a positive reaction with A/California/04/2009

(H1N1) and A/Wisconsin/WSLH049/09 (H1N1) viruses, whereas A/Osaka/164/09 (H1N1) was more readily detected by some IRDTs. The overall trend of detection was consistent among the IRDTs. Since these three isolates were propagated in MDCK cells under the same conditions and share 99.8%-100% amino acid sequence homology in NP, it is not clear why there were differences in sensitivity among the virus strains.

Finally, we evaluated the detection sensitivity of the IRDTs to pandemic (H1N1) 2009 viruses in our clinical specimens. Specifically, we used nasal swabs collected from three patients infected with pandemic (H1N1) 2009 influenza viruses (Table 3). Of the nine IRDTs tested, ESPLINE® Influenza A & B-N was the most sensitive for detecting pandemic viruses in our clinical specimens, as was the case with most of the viruses in cell culture fluids tested above (Table 9). Importantly, some IRDTs failed to detect any viral antigens even with clinical specimens containing more than $10^5$ pfu/ml of virus. These results suggest that the IRDTs tested in this study can be used to diagnose influenza caused by pandemic (H1N1) 2009 viruses, although some of the IRDTs tested may not be suitable for this purpose especially at an early stage of infection when the virus load is relatively low.

Here, we investigated the detection sensitivity of 20 IRDTs to various isolates of influenza A and B viruses. Although most of the IRDTs examined in this study detected not only seasonal, but also human and avian H5N1, other non-H5N1 avian, and swine-origin pandemic (H1N1) 2009 viruses within 30 min, our data indicate that the detection sensitivity of some IRDTs is suboptimal for application to virus strains other than the currently circulating seasonal viruses as demonstrated elsewhere (7, 24, 27). The Centers

for Disease Control and Prevention (CDC) has also reported that IRDTs often fail to positively identify patients infected with pandemic (H1N1) 2009 viruses (2). Specimens collected early in the course of influenza infection can be mislabeled as negative if the amount of NP they contain is insufficient to be detected by the IRDT. In fact, several patients have not been administered influenza drugs because of negative results at the first visit, only to be later diagnosed with pandemic H1N1 influenza infection (17). Such delays in the administration of antiviral treatment can increase the severity of the infection and even the likelihood of mortality. As long as the fundamental detection principle of IRDTs is based on an antigen-antibody reaction with antibody(s) against a viral protein, this limitation to the sensitivity of IRDTs will persist. Perhaps it is time to develop new IRDTs that target alternative viral components, such as viral RNA.

**Acknowledgements**

We thank Susan Watson for editing this manuscript. This work was supported, in part, by Grants-in-Aid for Specially Promoted Research and for Scientific Research, by a Contract Research Fund for the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases, by ERATO (Japan Science and Technology Agency), by the Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by National Institute of Allergy and Infectious Diseases Public Health Service research grants, USA. MO was partly supported by a Japan Society for the Promotion of Science Postdoctoral Fellowship for Research Abroad.

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**Table 1. Influenza rapid diagnostic tests examined.**

| IRDT | Manufacturer | Time (min)[a] | Dilution rate (%)[b] | Format[c] | Storage temperature (°C) | Shelf life (month) |
|---|---|---|---|---|---|---|
| ESPLINE® Influenza A & B-N | Fujirebio Inc. | 15 | 7.5 | Well | 1-30 | 15 |
| Directigen™ Flu A+B Test Kit | Beckton, Dickinson and Co. | 5 | 37.5 | Well | 2-25 | 12 |
| RapidTesta® FLU stick | Sekisui Medical Co., Ltd. | 3-10 | 100 | Test strip | 2-30 | 24 |
| Clearview® Exact Influenza A&B | Inverness Medical Japan Co., Ltd. | 15 | 100 | Test strip | 2-30 | 24 |
| QuickEx-Flu SEIKEN | Denka Seiken Co., Ltd. | 8 | 100 | Test strip | 2-30 | 12 |
| QUICKVUE® Rapid SP Influenza test | DS Pharma Biomedical Co., Ltd. | 10 | 100 | Test strip | 2-30 | 24 |
| POCTEM® S Influenza | Sysmex Co. | 10 | 22.0 | Test strip | 2-30 | 18 |
| Statmark™ FLU Stick A/B | Nichirei Biosciences Inc. | 10 | 100 | Test strip | 2-30 | 24 |
| PURORASUTO Flu | Mitsubishi Chemical Medience Co. | 10 | 16.7 | Well | 1-30 | 17 |
| BD Flu Examan™ | Beckton, Dickinson and Co. | 15 | 15.0 | Well | 2-25 | 18 |
| RapidTesta® Flu II | Sekisui Medical Co., Ltd. | 5-15 | 19.2 | Well | 2-30 | 24 |
| Quick Chaser® Flu A,B | Mizuho Medy Co., Ltd. | 15 | 20.8 | Well | 1-30 | 24 |
| Capilia® Flu A,B | Alfresa Co. | 15 | 9.1 | Well | 2-30 | 21 |
| CHECK Flu A·B | Rohto Pharmaceutical Co., Ltd | 15 | 20.0 | Well | 2-30 | 21 |
| ImmunoAce Flu | Tauns Laboratories, Inc. | 3-15 | 12.5 | Well | 2-30 | 18 |
| QuickNavi™ Flu | Denka Seiken Co., Ltd. | 8 | 12.0 | Well | 2-30 | 15 |
| POCTEM® Influenza A/B | Sysmex Co. | 15 | 22.2 | Well | 2-30 | 18 |
| Statmark™ InfluenzaA/B | Nichirei Bioscience Inc. | 15 | 11.3 | Well | 1-30 | 18 |
| SpotCem™ i-Line FluAB | Arkray, Inc. | 5-15 | 15.0 | Well | 2-30 | 18 |
| Immunotrap InfluenzaA·B | Shino-Test Co. | 1 | 17.0 | Well | 2-8 | 18 |

[a]Time required to obtain test results is according to the manufacturer's instructions.

[b]For all IRDTs examined, the test specimen (A) was required 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: volume C / (volume A + volume B) x 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, and Test strip format; the test strip is dipped into the diluted specimens and the reaction occurs on the strip.

**Table 2. Influenza virus strains tested.**

| Category[a] | Virus strain | Subtype or type[b] | | Host | Abbreviation[c] |
|---|---|---|---|---|---|
| Seasonal viruses | A/Yokohama/58/06 | H1N1 | | Human | Seasonal H1N1-1 |
| | A/Kawasaki/UT-K23/08 | | | Human | Seasonal H1N1-2 |
| | A/Kawasaki/UT-K25/08 | | | Human | Seasonal H1N1-3 |
| | A/Kawasaki/UTK-4/09 | | | Human | Seasonal H1N1-4 |
| | A/Yokohama/P2869/05 | H3N2 | | Human | Seasonal H3N2-1 |
| | A/Tokyo/UT-SK1/07 | | | Human | Seasonal H3N2-2 |
| | A/Kawasaki/UT-K20/08 | | | Human | Seasonal H3N2-3 |
| | B/Yokohama/P2922/05 | Type B | | Human | Seasonal type B |
| | B/Tokyo/UT-E2/08 | | | Human | Seasonal type B |
| Highly pathogenic H5N1 viruses | A/Hong Kong/483/97 | H5N1 | 3 | Human | Human H5N1-1 |
| | A/Hong Kong/212/03 | | 1 | Human | Human H5N1-2 |
| | A/Hong Kong/213/03 | | 1 | Human | Human H5N1-3 |
| | A/Vietnam/UT3040/04 | | 1 | Human | Human H5N1-4 |
| | A/Vietnam/UT30850/05 | | 2.3.4 | Human | Human H5N1-5 |
| | A/Indonesia/UT3006/05 | | 2.1.3 | Human | Human H5N1-6 |
| | A/chicken/Indonesia/UT1001/03 | | 2.1.1 | Chicken | Avian H5N1-1 |
| | A/chicken/Kyoto/3/04 | | 2.5 | Chicken | Avian H5N1-2 |
| | A/chicken/Vietnam/UT-G04/05 | | 1 | Chicken | Avian H5N1-3 |
| | A/chicken/Indonesia/UT2094/05 | | 2.1.3 | Chicken | Avian H5N1-4 |
| | A/duck/Vietnam/5001/05 | | 1 | Duck | Avian H5N1-5 |
| | A/duck/Vietnam/TY80/06 | | 2.3.4 | Duck | Avian H5N1-6 |
| | A/chicken/Vietnam/TY62/06 | | 2.3.2 | Chicken | Avian H5N1-7 |
| | A/whooper swan/Mongolia/2/06 | | 2.2 | Swan | Avian H5N1-8 |
| | A/chicken/Miyazaki/K11/07 | | 2.2 | Chicken | Avian H5N1-9 |
| | A/whooper swan/Akita/1/08 | | 2.3.2 | Swan | Avian H5N1-10 |
| Other avian viruses | A/chicken/Ibaraki/1/05 | H5N2 | | Chicken | Avian H5N2 |
| | A/tern/South Africa/ | H5N3 | | Tern | Avian H5N3 |
| | A/chicken/Hong Kong/G9/97 | H9N2 | | Chicken | Avian H9N2 |
| | A/chicken/Japan/25 | H7N7 | | Chicken | Avian H7N7 |
| Pandemic (H1N1) 2009 viruses | A/California/04/09 | H1N1 | | Human | Pandemic 2009-1 |
| | A/Wisconsin/WSLH049/09 | | | Human | Pandemic 2009-2 |
| | A/Osaka/164/09 | | | Human | Pandemic 2009-3 |

[a]Viruses tested are categorized into four groups based on their properties.

[b]As for H5N1 viruses, the clade of each strain is also shown.

[c]Abbreviation used in the following Tables is shown.

1 **Table 3. Clinical specimens analyzed.**

| Specimen ID | Virus titer[a] |
|---|---|
| MW | $1.0 \times 10^5$ pfu/ml |
| YT | $2.9 \times 10^5$ pfu/ml |
| AT | $2.1 \times 10^5$ pfu/ml |

2

3 [a]Virus titers [plaque-forming unit (pfu)] were determined in MDCK cells.

**Table 4. Detection sensitivity to seasonal virus strains[a].**

| IRDT | Minimum amount of virus for a positive reaction ($log_{10}$ $TCID_{50}$/100 µl) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Seasonal H1N1-1 | Seasonal H1N1-2 | Seasonal H1N1-3 | Seasonal H1N1-4 | Seasonal H3N2-1 | Seasonal H3N2-2 | Seasonal H3N2-3 | Seasonal type B-1 | Seasonal type B-2 |
| ESPLINE® Influenza A & B-N | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 5 | 4 |
| Directigen™ Flu A+B Test Kit | 4 | 3 | 3 | 4 | 5 | 5 | 4 | 6 | 4 |
| RapidTesta® FLU stick | 4 | 3 | 3 | 4 | 5 | 5 | 4 | 6 | 4 |
| Clearview® Exact Influenza A&B | 3 | 4 | 3 | 4 | 4 | 5 | 3 | 4 | 3 |
| QuickEx-Flu SEIKEN | 3 | 3 | 3 | 4 | 5 | 4 | 4 | 6 | 5 |
| QUICKVUE® Rapid SP Influenza test | 4 | 3 | 3 | 4 | 5 | 5 | 4 | 5 | 4 |
| POCTEM® S Influenza | 4 | 4 | 4 | 4 | 5 | 5 | 4 | 6 | 4 |
| Statmark™ FLU Stick A/B | 4 | 4 | 4 | 5 | 5 | 5 | 4 | >6 | 5 |
| PURORASUTO Flu | 3 | 3 | 3 | 4 | 5 | 5 | 3 | 6 | 4 |
| BD Flu Examan™ | 3 | 3 | 3 | 4 | 5 | 5 | 4 | 6 | 5 |
| RapidTesta® Flu II | 4 | 4 | 4 | 4 | 5 | 5 | 4 | 6 | 4 |
| Quick Chaser® Flu A,B | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 5 | 4 |
| Capilia® Flu A,B | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 6 | 5 |
| CHECK Flu A·B | 4 | 4 | 4 | 5 | 6 | 5 | 4 | 6 | 4 |
| ImmunoAce Flu | 4 | 4 | 4 | 4 | 5 | 5 | 4 | 6 | 4 |
| QuickNavi™ Flu | 3 | 3 | 3 | 4 | 5 | 5 | 4 | 6 | 5 |
| POCTEM® Influenza A/B | 4 | 4 | 4 | 5 | 5 | 5 | 4 | 6 | 5 |
| Statmark™ InfluenzaA/B | 4 | 4 | 5 | 5 | 6 | 6 | 5 | >6 | 5 |
| SpotCem™ i-Line FluAB | 5 | 6 | 6 | 6 | 6 | 6 | 5 | >6 | 5 |
| Immunotrap InfluenzaA·B | 4 | 5 | 5 | 5 | 6 | 6 | 4 | 6 | 6 |

[a] $10^1$ to $10^6$ $TCID_{50}$ of viruses in 100 µl aliquots of culture supernatants or allantoic fluid were subjected to IRDTs. Detection sensitivity is shown by the detection limit of the virus titer ($log_{10}$ $TCID_{50}$/100 µl).

**Table 5. Detection sensitivity to human H5N1 virus strains[a].**

| IRDT | Minimum amount of virus for a positive reaction ($log_{10}$ $TCID_{50}$/100 µl) | | | | | |
|---|---|---|---|---|---|---|
| | Human H5N1-1 | Human H5N1-2 | Human H5N1-3 | Human H5N1-4 | Human H5N1-5 | Human H5N1-6 |
| ESPLINE® Influenza A & B-N | 5 | 3 | 4 | 4 | 4 | 4 |
| Directigen™ Flu A+B Test Kit | 5 | 3 | 4 | 5 | 4 | 4 |
| RapidTesta® FLU stick | 6 | 4 | 5 | 5 | 5 | 5 |
| Clearview® Exact Influenza A&B | 6 | 4 | 5 | 6 | 5 | 5 |
| QuickEx-Flu SEIKEN | 5 | 3 | 4 | 5 | 4 | 4 |
| QUICKVUE® Rapid SP Influenza test | 5 | 3 | 4 | 5 | >6 | 5 |
| POCTEM® S Influenza | 6 | 4 | 5 | 5 | 5 | 5 |
| Statmark™ FLU Stick A/B | >6 | 6 | >6 | >6 | >6 | >6 |
| PURORASUTO Flu | 5 | 3 | 4 | 5 | 4 | 4 |
| BD Flu Examan™ | 5 | 3 | 4 | 5 | 4 | 5 |
| RapidTesta® Flu II | 6 | 3 | 5 | 5 | 4 | 4 |
| Quick Chaser® Flu A,B | 6 | 4 | 5 | 5 | 5 | 5 |
| Capilia® Flu A,B | 6 | 4 | 5 | 6 | 5 | 5 |
| CHECK Flu A·B | >6 | >6 | >6 | >6 | >6 | >6 |
| ImmunoAce Flu | 6 | 4 | 5 | 5 | 6 | 5 |
| QuickNavi™ Flu | 6 | 3 | 4 | 5 | 4 | 4 |
| POCTEM® Influenza A/B | 6 | 4 | 5 | 5 | 5 | 6 |
| Statmark™ InfluenzaA/B | >6 | 5 | 6 | >6 | 6 | 6 |
| SpotCem™ i-Line FluAB | >6 | 5 | 6 | >6 | 6 | >6 |
| Immunotrap InfluenzaA·B | 6 | 5 | 5 | 6 | 6 | 5 |

[a]Experiments were performed as described in the legend to Table 4.

**Table 6. Detection sensitivity to avian H5N1 virus strains[a].**

| IRDT | Minimum amount of virus for a positive reaction ($log_{10}$ $TCID_{50}$/100 µl) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Avian H5N1-1 | Avian H5N1-2 | Avian H5N1-3 | Avian H5N1-4 | Avian H5N1-5 | Avian H5N1-6 | Avian H5N1-7 | Avian H5N1-8 | Avian H5N1-9 | Avian H5N1-10 |
| ESPLINE® Influenza A & B-N | 2 | 3 | 3 | 4 | 5 | 4 | 4 | 5 | 5 | 5 |
| Directigen™ Flu A+B Test Kit | 3 | 3 | 3 | 5 | 5 | 4 | 4 | 5 | 5 | 5 |
| RapidTesta® FLU stick | 3 | 3 | 3 | 5 | 6 | 5 | 5 | 5 | 5 | 6 |
| Clearview® Exact Influenza A&B | 4 | 4 | 4 | 6 | 6 | 6 | 5 | 6 | 6 | 6 |
| QuickEx-Flu SEIKEN | 3 | 4 | 3 | 5 | 5 | 4 | 4 | 5 | 5 | 5 |
| QUICKVUE® Rapid SP Influenza test | 3 | 3 | 3 | 5 | 5 | 5 | 4 | 5 | 5 | 5 |
| POCTEM® S Influenza | 4 | 4 | 4 | 5 | 6 | 5 | 5 | 6 | 5 | 6 |
| Statmark™ FLU Stick A/B | >6 | 5 | 5 | >6 | >6 | >6 | >6 | >6 | >6 | >6 |
| PURORASUTO Flu | 3 | 3 | 3 | 4 | 5 | 4 | 4 | 5 | 5 | 5 |
| BD Flu Examan™ | 3 | 3 | 3 | 4 | 5 | 4 | 4 | 5 | 5 | 5 |
| RapidTesta® Flu II | 3 | 4 | 3 | 5 | 6 | 5 | 5 | 5 | 5 | 6 |
| Quick Chaser® Flu A,B | 4 | 4 | 4 | 5 | 6 | 5 | 5 | 6 | 6 | 6 |
| Capilia® Flu A,B | 5 | 4 | 4 | 6 | 6 | 5 | 6 | 5 | 6 | 6 |
| CHECK Flu A·B | >6 | >6 | >6 | >6 | >6 | >6 | >6 | >6 | >6 | >6 |
| ImmunoAce Flu | 4 | 4 | 4 | 5 | 6 | 5 | 5 | 6 | 5 | 6 |
| QuickNavi™ Flu | 3 | 4 | 4 | 5 | 5 | 4 | 5 | 5 | 5 | 6 |
| POCTEM® Influenza A/B | 3 | 4 | 4 | 6 | 6 | 6 | 5 | 6 | 6 | 6 |
| Statmark™ InfluenzaA/B | 6 | 6 | 6 | >6 | >6 | 6 | 6 | >6 | >6 | >6 |
| SpotCem™ i-Line FluAB | 6 | 5 | 5 | 6 | >6 | 6 | 6 | 6 | >6 | >6 |
| Immunotrap InfluenzaA·B | 4 | 4 | 5 | 6 | >6 | 6 | 6 | 6 | 6 | >6 |

[a]Experiments were performed as described in the legend to Table 4.

**Table 7. Detection sensitivity to other subtypes of avian viruses[a].**

| IRDT | Minimum amount of virus for a positive reaction ($log_{10}$ $TCID_{50}$/100 µl) | | | |
|---|---|---|---|---|
| | Avian H5N2 | Avian H5N3 | Avian H9N2 | Avian H7N7 |
| ESPLINE® Influenza A & B-N | 5 | 4 | 4 | 3 |
| Directigen™ Flu A+B Test Kit | 5 | 4 | 4 | 4 |
| RapidTesta® FLU stick | 5 | 5 | 4 | 4 |
| Clearview® Exact Influenza A&B | 6 | 4 | 5 | 5 |
| QuickEx-Flu SEIKEN | 5 | 4 | 4 | 4 |
| QUICKVUE® Rapid SP Influenza test | 5 | 4 | 4 | 4 |
| POCTEM® S Influenza | 5 | 5 | 5 | 4 |
| Statmark™ FLU Stick A/B | 5 | 5 | 5 | 6 |
| PURORASUTO Flu | 5 | 4 | 4 | 4 |
| BD Flu Examan™ | 5 | 4 | 4 | 4 |
| RapidTesta® Flu II | 5 | 5 | 4 | 4 |
| Quick Chaser® Flu A,B | 6 | 5 | 5 | 5 |
| Capilia® Flu A,B | 5 | 5 | 5 | 5 |
| CHECK Flu A·B | >6 | 6 | >6 | >6 |
| ImmunoAce Flu | 5 | 5 | 4 | 4 |
| QuickNavi™ Flu | 5 | 4 | 4 | 4 |
| POCTEM® Influenza A/B | 5 | 5 | 5 | 4 |
| Statmark™ InfluenzaA/B | >6 | 6 | 6 | 6 |
| SpotCem™ i-Line FluAB | 6 | 6 | 6 | 5 |
| Immunotrap InfluenzaA·B | 6 | 5 | 6 | 5 |

[a]Experiments were performed as described in the legend to Table 4.

**Table 8. Detection sensitivity to 2009 H1N1 viruses[a].**

| IRDT | Minimum amount of virus for a positive reaction ($log_{10}$ $TCID_{50}$/100 µl) | | |
|---|---|---|---|
| | Pandemic 2009-1 | Pandemic 2009-2 | Pandemic 2009-3 |
| ESPLINE® Influenza A & B-N | 4 | 4 | 3 |
| Directigen™ Flu A+B Test Kit | 5 | 4 | 3 |
| RapidTesta® FLU stick | 4 | 4 | 3 |
| Clearview® Exact Influenza A&B | 5 | 5 | 4 |
| QuickEx-Flu SEIKEN | 5 | 4 | 3 |
| QUICKVUE® Rapid SP Influenza test | 5 | 4 | 3 |
| POCTEM® S Influenza | 5 | 5 | 4 |
| Statmark™ FLU Stick A/B | 6 | 6 | 5 |
| PURORASUTO Flu | 5 | 4 | 3 |
| BD Flu Examan™ | 5 | 4 | 3 |
| RapidTesta® Flu II | 5 | 5 | 3 |
| Quick Chaser® Flu A,B | 5 | 5 | 4 |
| Capilia® Flu A,B | 6 | 5 | 4 |
| CHECK Flu A·B | 6 | 5 | 4 |
| ImmunoAce Flu | 5 | 4 | 3 |
| QuickNavi™ Flu | 5 | 5 | 4 |
| POCTEM® Influenza A/B | 6 | 5 | 4 |
| Statmark™ InfluenzaA/B | 6 | 6 | 5 |
| SpotCem™ i-Line FluAB | 6 | 6 | 5 |
| Immunotrap InfluenzaA·B | 6 | 5 | 5 |

[a]Experiments were performed as described in the legend to Table 4.

**Table 9. Sensitivity of IRDTs for the detection of 2009 H1N1 viruses in nasal swabs.**

| IRDT[a] | Format[a] | Maximum dilution of specimen for a positive reaction[b] | | |
|---|---|---|---|---|
| | | MW | YT | AT |
| ESPLINE® Influenza A & B-N | Well | 2 | 20 | 2 |
| BD Flu Examan™ | Well | 2 | 2 | 2 |
| Quick Chaser® Flu A,B | Well | <2 | 2 | 2 |
| Capilia® Flu A,B | Well | <2 | <2 | <2 |
| Statmark™ FLU Stick A/B | Well | <2 | <2 | <2 |
| SpotCem™ i-Line FluAB | Well | <2 | <2 | <2 |
| RapidTesta® FLU stick | Test strip | 2 | 2 | 2 |
| Clearview® Exact Influenza A&B | Test strip | <2 | <2 | <2 |
| Statmark™ FLU Stick A/B | Test strip | <2 | <2 | <2 |

[a]Six representative well format and three representative test strip format IRDTs were evaluated for their sensitivities for detecting pandemic H1N1 viruses in clinical specimens.

[b]Specimens were diluted 2-, 20-, and 200-fold and subjected to IRDTs.