EVALUATION OF 11 FDA-APPROVED RAPID INFLUENZA DIAGNOSTIC TESTS USING 23 RECENTLY CIRCULATING INFLUENZA VIRUSES

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INTRODUCTION

Rapid influenza diagnostic tests (RIDTs) continue to play an important role in clinical diagnosis given their quick turnaround time, low cost and ease of use (1). Although commercially available antigen detection influenza assays vary widely in their reported sensitivities, their high specificities and positive predictive values during peak season, facilitate timely treatment decisions and enable improved patient care by limiting additional diagnostic and therapeutic interventions (including hospitalizations)(2-6). Furthermore, their reduced complexity is particularly useful in low resource settings both in the U.S. and abroad.

Despite advantages many uncertainties exist with describing and comparing sensitivities of RIDTs . Only a few published studies compare multiple RIDTs side by side (often no more than three tests in the same study). Existing studies use a wide variety of sample types, protocols, and influenza viruses making it difficult to compare results across studies. Many of the studies (including those in product inserts) were completed during seasons when influenza strains circulating in the general population are no longer relevant.

The purpose of this study was to assess the analytical variability of all FDA-cleared RIDTs with a set of recently circulating viruses. Additionally, we evaluated the reproducibility of RIDT results within our laboratory.

Here we show a comparison of the reactivity of each RIDT available in the U.S during early 2011 with 25 influenza viruses including: 4 H1N1 2009 pandemic influenza A strains, 6 H1N1 seasonal influenza A strains, 6 H3N2 influenza A strains, 2 recombinant influenza A vaccine strains, 3 Yamagata-lineage influenza B strains, and 4 Victoria-lineage influenza B strains. Additionally, reproducibility is shown with a subset of test kits at three separate time points within the MCW laboratory.

MATERIALS AND METHODS

Sample Preparation: Each of the 25 viruses (Table 1) used in this experiment were diluted in 0.9% saline solution (Sigma-Aldrich Co., St. Louis, MO) at ratios of 1:10 (10^{-1}), 1:31.62 ($10^{-1.5}$), 1:100 (10^{-2}), 1:316.2 ($10^{-2.5}$), and 1:1000 (10^{-3}). Briefly, after virus stock dilution in saline, 50 µl of each dilution was placed into 1.5 ml microcentrifuge tubes (three for each virus dilution and RIDT test kit) and stored on ice. At the time of testing, a sterile foam swab (Catalog # 25-1506-1PF, Puritan Medical Products Co. LLC, Guilford, ME) was used to absorb all 50 µl from one microcentrifuge tube (visually inspected). These swabs served as the input for each of the RIDTs following the manufacturer's test protocol for a swab sample. This approach was modified for use with the SAS RIDTs liquid sample procedure, as the SAS test kits are not indicated for testing swab samples.

Determination and Documentation of RIDT Results: Observed reactivity (presence/absence of a test line) or instrument output for the 3M RIDT was documented for each sample tested. Technicians recorded the result for influenza A, influenza B, and the internal control as positive, negative, or invalid. When all testing was completed for the day, the RIDT results were entered into an electronic worksheet and verified by a different technician to ensure accuracy.

ELISA Detection of influenza nucleoprotein in virus dilution samples: In order to confirm the presence of influenza A nucleoprotein (IAV) in each sample, an aliquot of each dilution was tested with the Influenza A or B Virus Nucleoprotein Antigen Capture ELISA (Catalog # IAV-142 or IBV-221, Virusys Corporation, Taneytown, MD), following the manufacturer's instructions with the following exception: instead of combining 200 µl of virus dilution with 50 µl of IAV sample preparation buffer and loading 100 µl of sample onto the plate, we combined 100 µl of each virus dilution with 25 µl of IAV sample preparation reagents and loaded 100 µl onto the IAV Antigen Capture Plate, to decrease the waste of reagents. The absorbance readings at 450 nm (A_{450}) were recorded and averaged between ELISA replicates at each virus dilution.

Detection of Influenza A and B Matrix Gene RNA by Real-Time RT-PCR: To ensure that all virus dilutions were prepared appropriately, real-time RT-PCR (rRT-PCR) testing was performed with 100 µl of each virus stock and dilution. , using the CDC Real-Time RT-PCR (rRT-PCR) Protocol for Detection and Characterization of Influenza (Version 2007), the NucliSENS® easyMAG™ System (bioMérieux, Durham, NC) using the off-board lysis protocol with elution in 100µl, the Ambion AgPath-ID™ One-Step RT-PCR Kit (Catalog # AM1005, Invitrogen Corporation, Carlsbad, CA), and the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Three 5 µl samples of extracted RNA for each dilution were tested according to the CDC's testing protocol. Microsoft Excel was used to plot the points on an XY scatter plot (with the average Ct value of the rRT-PCR replicates plotted on the X axis vs. the $CEID_{50}$/ml concentration plotted on the Y axis).

Reproducibility Testing: Reproducibility testing was performed using a subset (five) of the 11 RIDT kits in an identical manner as described above. Samples were diluted from new stocks of virus that hadn't been thawed previously.

Table 1. Viruses Used in Evaluating 11 FDA Approved RIDTs

Virus Number	Virus Name	Type/ Subtype	Stock Concentration ($CEID_{50}$/ml)
1	A/California/7/2009 NYMC X-179A	A/H1N1pdm	1.4×10^9
2	A/Hong Kong/2652/2006	A/H1N1	1.58×10^9
3	A/Brisbane/10/2007	A/H3N2	3.9×10^7
4	A/California/7/2009	A/H1N1pdm	5.2×10^7
5	A/Cambodia/371/2007	A/H1N1	2.7×10^8
6	A/Florida/3/2006	A/H1N1	1.4×10^8
7	A/Perth/16/2009	A/H3N2	1.1×10^8
8	A/California/8/2009	A/H1N1pdm	1.6×10^8
9	A/Wisconsin/15/2009	A/H3N2	5.8×10^6
10	A/South Dakota/6/2007	A/H1N1	7.9×10^8
11	A/Mexico/4108/2009	A/H1N1pdm	2.2×10^7
12	A/Santiago/7981/2006	A/H3N2	8.1×10^8
13	A/New York/18/2009	A/H1N1pdm	1.6×10^8
14	A/Uruguay/716/2007	A/H3N2	8.9×10^8
15	A/Brisbane/59/2007	A/H1N1	1.6×10^9
16	A/Solomon Islands/3/2006	A/H1N1	2.5×10^8
17	A/Henan/Jinshui/147/2007	A/H3N2	5.0×10^8
18	A/Uruguay/716/2007 X175C	A/H3N2	6.9×10^8
19	B/Bangladesh/5278/2006	B/Victoria	2.84×10^8
20	B/Pennsylvania/5/2007	B/Victoria	2.2×10^8
21	B/Brisbane/3/2007	B/Yamagata	1.8×10^7
22	B/Florida/4/2006	B/Yamagata	6.95×10^8
23	B/Brisbane/60/2008	B/Victoria	1.0×10^8
24	B/Pennsylvania/7/2007	B/Yamagata	3.4×10^7
25	B/Victoria/304/2006	B/Victoria	8.9×10^8

Table 4. Reactivity of each RIDT by Influenza Type and Subtype

	Flu A – 2009 H1N1 Pandemic Strains[2]					Flu A – Vaccine Reassortant Strains[3]					Flu A – H1N1 Seasonal Strains[4]					Flu A – H3N2 Strains[5]					Flu B Strains[6]				
	$10^{-1.0}$	$10^{-1.5}$	$10^{-2.0}$	$10^{-2.5}$	$10^{-3.0}$	$10^{-1.0}$	$10^{-1.5}$	$10^{-2.0}$	$10^{-2.5}$	$10^{-3.0}$	$10^{-1.0}$	$10^{-1.5}$	$10^{-2.0}$	$10^{-2.5}$	$10^{-3.0}$	$10^{-1.0}$	$10^{-1.5}$	$10^{-2.0}$	$10^{-2.5}$	$10^{-3.0}$	$10^{-1.0}$	$10^{-1.5}$	$10^{-2.0}$	$10^{-2.5}$	$10^{-3.0}$
SAS FluAlert Influenza A Test; SAS FluAlert Influenza B Test	3	0	0	0	0	6	6	0	0	0	15	9	0	0	0	18	6	0	0	0	21	21	9	0	0
SAS FluAlert Influenza A & B Test[1]	6	0	0	0	0	3	0	0	0	0	3	0	0	0	0	3	0	0	0	0	21	18	3	0	0
3M™ Rapid Detection Flu A+B Test	12	9	0	0	0	6	6	6	0	0	18	13	7	0	0	18	16	10	0	0	21	21	19	5	0
BinaxNOW Influenza A & B Test	12	0	0	0	0	6	6	0	0	0	12	6	0	0	0	15	0	0	0	0	20	9	0	0	0
X/pect® Flu A&B	9	0	0	0	0	6	6	0	0	0	18	11	3	0	0	18	9	3	0	0	21	21	15	0	0
TRUFLU Test	12	9	0	0	0	6	6	6	0	0	18	12	3	0	0	18	18	6	0	0	21	18	5	0	0
OSOM Influenza A&B Test	12	9	0	0	0	6	6	3	0	0	15	9	4	0	0	18	9	0	0	0	21	18	2	0	0
QuickVue Influenza A+B Test[1]	12	12	0	0	0	6	6	6	0	0	18	12	9	0	0	18	18	12	0	0	21	21	21	3	0
QuickVue Influenza Test[1]	12	9	0	0	0	6	6	3	0	0	18	12	9	0	0	18	18	6	0	0	21	21	18	3	0
Directigen EZ Flu A+B (throat swab protocol)	12	12	9	0	0	6	6	6	0	0	18	18	9	0	0	18	18	9	0	0	21	21	3	0	0
Status® Flu A + B Test	12	9	3	0	0	6	6	3	0	0	18	18	9	3	0	18	15	9	3	0	18	15	9	0	0
% reactivity within group at each dilution	100%		99 – 70%		69 – 40%		39 – 10%		9 – 0%																

[1] CLIA Waived
[2] Four 2009 H1N1pdm influenza viruses listed in Table 1; each with 3 samples at each dilution (12 possible reactive samples for each dilution)
[3] Two reassortant influenza viruses listed in Table 1; each with 3 samples at each dilution (6 possible reactive samples for each dilution)
[4] Six H1N1 influenza viruses listed in Table 1; each with 3 samples at each dilution (18 possible reactive samples for each dilution)
[5] Six H3N2 influenza viruses listed in Table 1; each with 3 samples at each dilution (18 possible reactive samples for each dilution)
[6] Seven influenza B viruses (Victoria & Yamagata lineages) listed in Table 1; each with 3 samples at each dilution (21 possible reactive samples for each dilution)

Table 2. Rapid Influenza Diagnostic Test Properties/Protocols

RIDT	Manufacturer	Protocol/ Protocol	CLIA Status	Incubation Time	Exceptions to Standard Testing Approach
QuickVue Influenza Test	Quidel Corporation	Nasal Swab Procedure	Waived	10 min	-Used Supplied Foam Swab[1]
QuickVue Influenza A+B Test	Quidel Corporation	Nasal/ Nasopharyngeal Swab Procedure	Waived	1 min + 10 min	-Used Supplied Foam Swab[1]
3M™ Rapid Detection Flu A+B Test	3M Health Care	Nasopharyngeal Swap Sample Procedure	Moderate	15 min	-Specimens were prepared as for all tests, but then diluted in 0.5 ml of saline as per manufacturer's requirements
X/pect® Flu A&B	Remel, Inc.	swab specimens without dilution in transport media procedure	Moderate	15 min	
BinaxNOW Influenza A & B Test	Alere, Inc. /Inverness Medical	Nasopharyngeal and nasal swab elution using transport medium	Waived	15 min	
TRUFLU Test	Meridian Bioscience, Inc.	Nasal and nasopharyngeal swab specimens collected without transport medium procedure	Moderate	15 min	
OSOM Influenza A&B Test	Genzyme Corporation	Test Procedure	Moderate	10 min	-Used Supplied Foam Swab[1]
Directigen EZ Flu A+B	Becton, Dickinson and Company	Nasopharyngeal Wash/Aspirate and Nasopharyngeal Swab Specimens Procedure	Moderate	15 min	-Specimens were prepared as for all tests, but then diluted in 1 ml of saline as per manufacturer's requirements
Directigen EZ Flu A+B	Becton, Dickinson and Company	Throat Swab Procedure	Moderate	15 min	
SAS FluAlert Influenza A Test; SAS FluAlert Influenza B Test	SA Scientific, Ltd.	Test Procedure	Waived[2], but used Moderate Protocol	15 min	-No swab was used; instead 50 µl of virus was combined with 200 µl of saline to make the 250 µl sample required for the RIDT
SAS FluAlert Influenza A & B Test	SA Scientific, Ltd.	Nasal Washes or Aspirates Procedure	Moderate	15 min	-No swab was used; instead 50 µl of virus was combined with 200 µl of saline to make the 250 µl sample required for the RIDT
Status® Flu A + B Test	Princeton BioMeditech Corporation	Swab Specimen Procedure	Moderate	10-15 min	-Used Supplied Flocked Swab

[1]The manufacturer's supplied swabs are the same as those used for all other RIDTs
[2]The CLIA Moderate complexity protocol was used because the waived protocol caused a several invalid results during QC testing experiments
[3]Not required if using the CLIA waived protocol

Table 3. Ability of 11 FDA-Approved RIDTs to 25 Recently Isolated Influenza Viruses at any Concentration.

RIDT	# of Viruses Detected at Any Concentration					Viruses Not Detected	Total # of Tests	# of Invalid Tests
	Flu A – H1N1pdm	Flu A – H1N1seas[1]	Flu A – H3N2	Flu B – Victoria Lineage	Flu B – Yamagata Lineage			
3M™ Rapid Detection Flu A+B Test	4/4	8/8	6/6	4/4	3/3	N/A	453	0
BinaxNOW Influenza A & B Test[2]	4/4	6/8	5/6	4/4	3/3	6, 7, 16	351	1
Directigen EZ Flu A+B (throat swab protocol)	4/4	8/8	6/6	4/4	3/3	N/A	414	0
QuickVue Influenza A+B Test	4/4	8/8	6/6	4/4	3/3	N/A	456	0
Status® Flu A + B Test[2]	4/4	8/8	6/6	3/4	3/3	19	402	0
X/pect® Flu A&B[3]	3/4	8/8	6/6	4/4	3/3	8	405	2
OSOM Influenza A&B Test[4]	4/4	7/8	6/6	4/4	3/3	16	396	0
QuickVue Influenza Test[4]	4/4	8/8	6/6	4/4	3/3	N/A	441	0
SAS FluAlert Influenza A Test; SAS FluAlert Influenza B Test[4]	2/4	2/8	1/6	4/4	3/3	2, 4, 5, 6, 7, 9, 10, 11, 12, 14, 16, 17, 18	336	0
SAS FluAlert Influenza A & B Test[4]	1/4	7/8	6/6	4/4	3/3	4, 11, 13, 16	384	0
TRUFLU Test[4]	4/4	8/8	6/6	4/4	3/3	N/A	423	0

[1]For the purposes of this table the two influenza A reassortant viruses (#1 and #18) were considered seasonal H1N1 strains because they contain the NP gene from Influenza A/Puerto Rico/8/1934, which is a seasonal H1N1 virus
[2]All viruses not detected during the initial study were detected during reproducibility testing
[3]All viruses not detected during the initial study remained undetectable during reproducibility testing
[4]No reproducibility testing was done with this assay

Figure 1. Reproducibility of RIDTs with Influenza A Viruses

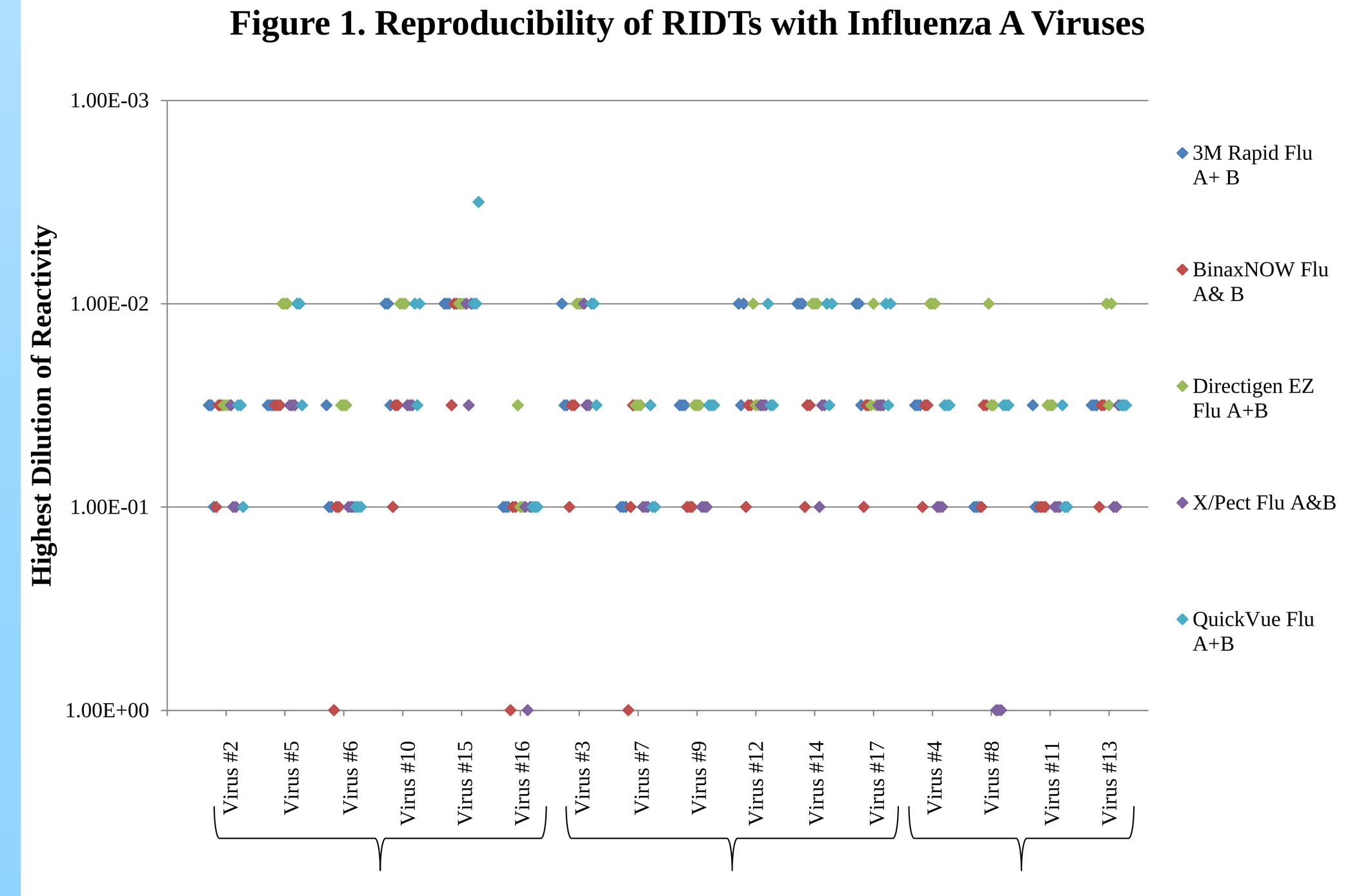
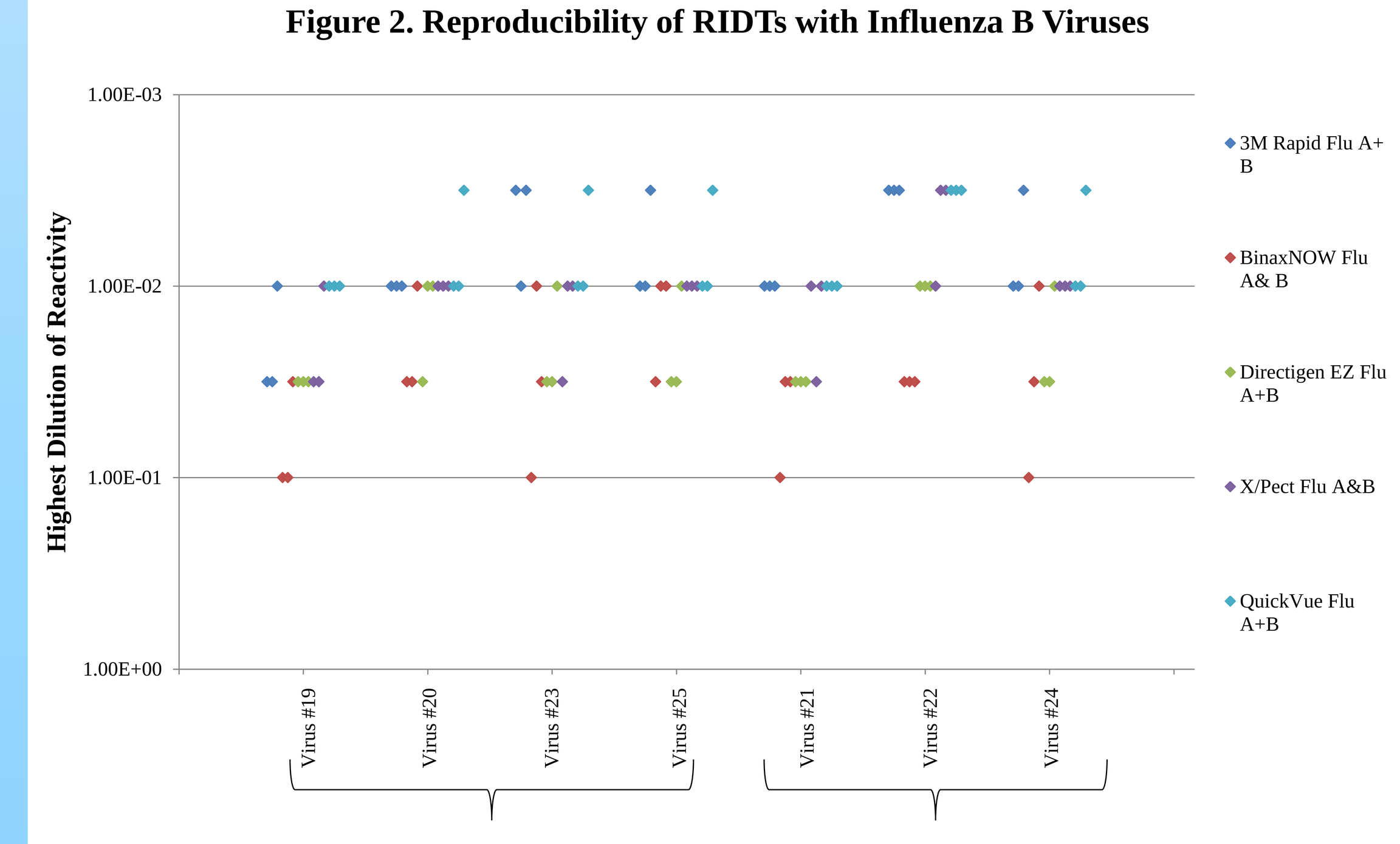


Figure 2. Reproducibility of RIDTs with Influenza B Viruses



RESULTS

Initial Study:

1) Of the 11 RIDTs evaluated, 5 detected all viruses at one or more dilutions (each with different virus concentration).
2) RIDTs were similarly reactive with 2009 H1N1 influenza A viruses, as with seasonal influenza A strains.
3) While most RIDTs demonstrated reactivity at the first dilution (highest virus concentration) for seasonal influenza A and B viruses, some test kits were less reactive with one or more viruses in each group; one test kit was less reactive across the influenza A viruses, but not with the Influenza B viruses.
4) Overall, the RIDTs were more reactive with influenza B viruses and showed less variation in reactivity levels for influenza B (with the exception of B/Bangladesh/5278/2006 (virus #19).
5) Lower levels of reactivity were not necessarily associated with less concentrated viral stocks (lower EID50/mL); Example: A/Perth/16/2009 (virus #7); B/Bangladesh/5278/2006 (virus #19).

Reproducibility Study (Figures 1 and 2):

1) The reactivity dilution for any virus/RIDT combination generally varied by no more than one half log dilution, with the exception of the BinaxNOW test which varied by more than one dilution with two viruses.
2) If the first set of testing is discounted, BinaxNOW variation was similar to other test kits with one consistent lot.
3) While different lots were generally used at different testing time points, only intra-laboratory variation could be assessed. Reproducibility within MCW would be optimum, given experienced techs who participated in the study.

DISCUSSION

This analytical comparison study using 23 recently isolated influenza viruses (and two recombinant influenza A viruses) and 11 FDA-approved RIDTs, suggests these tests as a whole are similarly reactive to 2009 H1N1 pandemic strains of influenza A as to seasonal H1N1 and H3N2 influenza A strains. While certain test kits appeared more reactive, only 2 test kits exceeded reactivity with more than three dilutions for any one virus in a virus group. This wider range was observed with Influenza B viruses for 3 test kits, and only one test kit for any Influenza A virus.

Repeated testing with 5 RIDTs suggests that RIDTs are generally reproducible within a half log dilution. While all test kit lots used were positive with the manufacturer-provided controls (data not shown), some test kit lots were more variable (e.g. one lot of the BinaxNOW test showed lower reactivity for almost all 25 viruses than other lots of the same test). Laboratories may consider preparing their own positive controls to allow detection of differences that wouldn't be observed when using the very concentrated manufacturer-provided control.

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