Evaluation of the Limit of Detection of the BD Veritor™ System Flu A+B Test and Two Rapid Influenza Detection Tests for Influenza Virus

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Background

Effective, potentially life-saving therapy with influenza antiviral therapy depends on diagnosis early in disease course. Influenza antiviral drugs are underutilized (CDC). Increased sensitivity of point-of-care influenza testing will increase opportunities for effective influenza antiviral use.

Objective

We sought to compare the limit of detection of three CLIA-waived rapid influenza detection tests for three strains of influenza virus that circulated in 2010-2011 or 2011-2012. The rapid tests were one instrument-based test, BD Veritor™ System for Flu A+B (BD Diagnostics), and two visual read tests, Binax NOW® Influenza A+B (Alere, Inc), and QuickVue® Influenza Test (Quidel, Inc).

Methods

Starting stock dilutions were influenza H1N1 A/California/7/2009 ($3.2x10^7$ $TCID_{50}$/mL), seasonal influenza H3N2 A/Brisbane 10/2007 ($4.6x10^7$ $TCID_{50}$/mL), and influenza B/Victoria/504/00 ($4.6x10^7$ $TCID_{50}$/mL).

- These strains were circulating in US in the 2010-2011 and/or 2011-2012 influenza seasons.
- Starting stock solutions were confirmed to be above the limit of detection for all testing methods.

Two-fold serial dilutions of each viral strain were prepared and samples were tested in triplicate by a single operator.

- Control samples were performed for each test following manufacturers instructions.
- The single operator that performed all rapid testing was blinded to sample concentration.

Testing of serial two-fold dilutions was performed for each rapid test until a negative test in triplicate was reached. From each two-fold dilution, RNA was extracted and RT-PCR was performed in triplicate.

- Influenza RT-PCR detection was performed using the "CDC Realtime rRTPCR Protocol for Detection and Characterization of Influenza."
- Cycle Threshold (CT) values of <40 are positive.

Results

H1N1 - A/California/7/2009

$TCID_{50}$/ml	BD			Binax			QV			CT PCR1	CT PCR2	CT PCR3
3.2×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	23.8	25.2	24.7
1.6×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	25.6	25.0	26.1
8.0×10^6	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	25.9	25.5	25.5
4.0×10^6	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	27.0	26.8	24.9
2.0×10^6	Pos	Pos	Pos				Neg	Neg	Neg	28.3	27.8	27.8
1.0×10^6	Pos	Pos	Pos							29.0	29.1	28.7
5.0×10^5	Pos	Pos	Pos							30.1	28.8	29.7
2.5×10^5	Pos	Pos	Pos							32.0	31.4	30.7
1.25×10^5	Neg	Neg	Neg							32.0	31.2	31.9

H3N2 - A/Brisbane/10/2007

$TCID_{50}$/ml	BD			Binax			QV			CT PCR1	CT PCR2	CT PCR3
4.6×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	21.9	21.5	21.2
2.3×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	23.0	23.1	23.3
1.2×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	24.2	24.6	24.6
5.8×10^6	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	25.9	26.1	25.8
2.9×10^6	Pos	Pos	Pos				Pos	Pos	Pos	26.6	27.3	26.7
1.4×10^6	Pos	Pos	Pos				Neg	Neg	Neg	26.4	26.5	26.5
7.2×10^5	Pos	Pos	Pos							27.9	28.5	27.9
3.6×10^5	Neg	Neg	Neg							29.3	28.8	29.0

B - B/Victoria/504/00

$TCID_{50}$/ml	BD			Binax			QV			CT PCR1	CT PCR2	CT PCR3
4.6×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	19.3	19.6	19.4
2.3×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	20.5	20.8	20.6
1.2×10^7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	21.8	22.2	22.4
5.8×10^6	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	22.9	23.2	23.3
2.9×10^6	Pos	Pos	Pos	Neg	Neg	Neg	Pos	Pos	Pos	23.1	23.4	22.6
1.4×10^6	Pos	Pos	Pos				Pos	Pos	Pos	23.6	24.1	24.8
7.2×10^5	Pos	Pos	Pos				Neg	Neg	Neg	25.1	25.5	25.5
3.6×10^5	Neg	Neg	Neg							25.6	25.6	27.0

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Results

All tests performed in triplicate were concordant (all positive or all negative) for each strain dilution. RT-PCR analysis was positive for each strain of influenza virus at the lowest concentration detected by any rapid test, confirming the presence of the expected influenza type.

Limit of Detection ($TCID_{50}$/mL) for influenza strains

	A(H1N1)	A(H3N2)	B
Binax NOW® Influenza A+B	$8x10^6$	$1.2x10^7$	$5.8x10^6$
QuickVue® Influenza	$4x10^6$	$2.9x10^6$	$1.4x10^6$
BD Veritor™ System Flu A+B	$2.5x10^5$	$7.2x10^5$	$7.2x10^5$

The BD Veritor Flu A+B test detected the lowest amount of influenza virus for all 3 strains.

Limitations

This study determined limits of detection for these tests using laboratory dilutions of circulating influenza strains. Clinical test sensitivity is measured using patient samples.

Conclusions

- The limit of detection for each rapid influenza detection test varied by influenza strain.
- The BD Veritor Flu A+B test had the lowest limit of detection for all three influenza strains, which was at least 2-fold and 8-fold lower than the QuickVue® Influenza and Binax NOW® Influenza A+B tests, respectively.
- A lower detection limit and an objective read by the BD Veritor™ System Flu A+B test may potentially impact clinical sensitivity, facilitate accurate influenza diagnosis and guide effective antiviral therapy.