

Comparison of Rapid Influenza Antigen Kits for Analytical Sensitivity and Compatibility with Viral Transport Medium.

Joseph A. Jollick, Nathan Chapman, and Mat Longiaru, PhD.

Diagnostic Hybrids • Athens, OH

ABSTRACT

INTRODUCTION: Influenza A and B cartridge kits serve a relevant purpose by providing a rapid answer regarding the presence or absence of the specific virus in a respiratory specimen. However, due to concerns regarding the kits sensitivity, prominent health organizations such as WHO and FDA strongly recommend that negative results generated by these kits be tested with a more sensitive method. Positive results may also benefit from culture confirmation, particularly when more than one virus may be present in the sample. Cell Culture is one of the accepted confirmation methods.

OBJECTIVE: We compared commercially available Rapid Influenza A and B kits for their analytical sensitivity to 5 Influenza A and 5 Influenza B isolates. The kits were also evaluated for their compatibility with available viral transport medium to determine the optimal Kit and Transport Medium combination so that a single swab from a patient will accommodate both the Rapid Kit and Cell Culture.

METHODS: For the analytical sensitivity comparison, we prepared dilutions of the isolates in a buffered saline solution. The Rapid Kits tested included BD Directigen™ EZ Flu A+B, Quidel QuickVue Influenza A + B Test, Remel X/pect™ FLU A&B and BinaxNOW®. Each of these were inoculated with each specimen dilution and examined for: level of detection, time to positivity and intensity of a positive band. R-Mix shell vials with coverslips were used as the cell culture system and processed with D3 Influenza A and B antibodies at 18 hours post inoculation. For the compatibility with viral transport medium study, we used the 2004 Influenza A and 2004 Influenza B isolates and Copan UTM and Remel M4-RT inoculated with 3 different virus levels. We also inoculated buffered saline solution with the same levels as a base line control. Un-inoculated vials of Copan UTM and Remel M4-RT were used as negative controls, as well as un-inoculated buffered saline. Each of these were tested with the 4 Rapid Kits mentioned above and then examined for: detection level compared to virus diluted in buffered saline, time to positivity, intensity of a positive band and occurrence of either false positives from the un-inoculated controls, or false negatives from the inoculated viral transport medium.

RESULTS: For each of the Rapid Kits, sensitivity to each Virus type and isolate was strain related. For Influenza A, BD Directigen EZ Kit was between double and up to 2 logs more sensitive than the other Kits. For Influenza B, BD and Quidel showed overall equivalent results with both being more sensitive than Remel and Binax. R-Mix results were not strain related and validated the need for culture by detecting each isolate to a level of 10 virus particles or lower. Overall, there was no significant difference in sensitivity performance with any of the Rapid Kits in conjunction with Copan UTM or Remel M4-RT; however, Quidel QuickVue showed a false positive Flu A band when used with M4-RT.

CONCLUSION: BD demonstrated the greatest sensitivity of the kits tested in this study. The virus titer required for the accurate detection was well above that required by the R-Mix culture system, validating the need for culture testing of negative antigen tests. Copan UTM proved to be compatible with all of the Rapid Kits indicating that it can be used for the rapid test and used for R-Mix culture if negative. Remel M4-RT has the same indications for use with all except Quidel.

STUDY OBJECTIVES

- Compare the analytical sensitivity of 4 prominent Influenza A and B Point of Care Tests (POCT) to R-Mix cell culture.
- Determine the performance characteristics of these POCT kits when PBS, M4 and UTM transport media are used as simulated, virus-spiked clinical specimens.
- Demonstrate influenza virus recovery over a 3-day storage period, and at two temperatures (ambient and 2-8°C), for UTM and UTM diluted with an equal volume of PBS to simulate a nasal aspirate or nasal wash.

REFERENCES

1. <http://www.fda.gov/cdrh/oivd/tips/rapidflu.html>
2. <http://www.cdc.gov/flu/professionals/labdiagnostics.htm>
3. http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en/
4. St. George, et al. J. of Clinical Virology Volume:24 Issue 1-2, Pg 107-115

MATERIALS AND METHODS

- **Working Virus Stocks.** PBS, and the commercially available transport media M4 (Remel) and UTM (Copan) were seeded with known amounts of influenza A or B virus from respiratory seasons covering 2001-2004. Non-seeded PBS, M4 and UTM were used as negative controls.
- **POCT Device Methods.** For POCT kits, the following was done according to their respective product inserts to effect sample treatment prior to application to the test device:
 - BD: 300 µL of specimen + 100 µL of lysis reagent.
 - 3 drops (~100 µL) were added to the device.
 - Binax: 100 µL of specimen directly onto device.
 - Quidel: 300 µL of specimen in test.
 - Note: Less than 100 µL travels up the device wick.
 - Remel: 100 µL of specimen + 100 µL of lysis reagent.
 - 200 µL was added to the device.

- All devices were examined between 15-30 min post-inoculation according to the instructions in the kit product insert. All devices were examined for:
- Analytical Sensitivity of isolates diluted in PBS.
 - Level of detection of isolates in UTM and M4 compared to PBS.
 - Appearance of false positive bands in the negative controls.
 - Inhibition of control bands.
- **R-Mix Cell Culture Confirmation Testing.** R-Mix monolayers were inoculated with 200 µL of each sample per monolayer and centrifuged at 700 x g for 1 hr and incubated at 35-37°C for 18 hrs. Monolayers were then fixed and stained with virus-specific monoclonal antibody labeled directly with FITC.
 - **Virus Recovery in UTM and UTM diluted 1:1 with Nasal Wash.** For virus recovery studies in UTM and diluted UTM, samples were taken at 24, 48 and 72 hrs after seeding the respective media and tested on R-Mix for % recovery.

RESULTS

Variable Analytical Sensitivity Among Commercial Kits by Flu Season

	2001 Flu A H3N2	2001 Flu B	2002 Flu A H3N2	2002 Flu B	2003 Flu A H3N2	2003 Flu B	2004 Flu A H3N2	2004 Flu B	Denver Flu A H1N1	Taiwan Flu B
BD	1200	500	1500	17000	80	7000	35	1200	550	18000
Binax	6000	5000	15000	17000	40000	70000	3500	1200	55000	180000
Quidel	1200	500	1500	1700	800	7000	350	120	5500	18000
Remel	1200	5000	15000	17000	40000	70000	350	1200	55000	18000

Figure 1: The table and the accompanying values in each square show the sensitivity of each of the four Rapid Antigen Flu A and B test kits. Gray squares represent the least sensitive test for each virus. Low passage clinical isolates from the prominent Flu A and B strains from the past 4 years were used, and 1 ATCC isolate of each A and B were used and diluted in PBS. Devices were inoculated according to each of their product inserts. R-Mix and R-Mix Too were used as Gold Standard cultures to confirm input levels for each virus at each dilution. These cultures were processed at 18 hrs. PI and both systems detected levels of virus at least 1 log, and usually 2-3 logs, lower than any of the kits. (Data not shown).

Compatibility of UTM (Copan) and M4 (Remel) with POCT Kits

		Copan UTM			Remel M4			PBS		
		Flu A	Flu B	NC	Flu A	Flu B	NC	Flu A	Flu B	NC
BD	High	+	+	-	+	+	-	+	+	-
	Med.	+	+	-	+	+	-	+	+	-
	Low	+	+	-	+	-	-	+	+	-
Binax	High	+	+	-	+	+	-	+	+	-
	Med.	-	+	-	-	+	-	-	+	-
	Low	-	-	-	-	-	-	-	-	-
Quidel	High	+	+	-	+?	+	+A	+	+	-
	Med.	+	+	-	+?	+	+A	+	+	-
	Low	+	+	-	+?	+	+A	-	+	-
Remel	High	+	+	-	+	+	-	+	+	-
	Med.	+	+	-	+	+	-	+	+	-
	Low	(+)	(+)	-	(+)	(+)	-	(+)	(+)	-

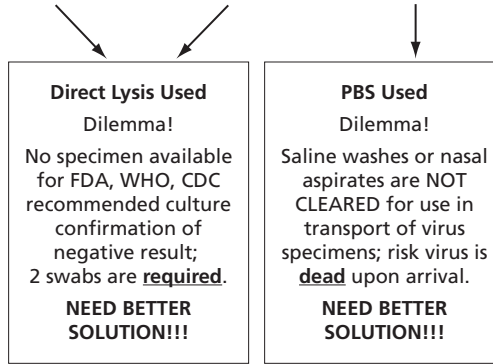
Table below shows input based on R-Mix processed at 18 hours PI for the levels of High, Med. And Low for Flu A and Flu B used in Figure 2

	FluA	FluB
High	33,000	1,600,000
Med.	3,300	160,000
Low	330	16,000

Figure 2: Influenza A, 2004 California strain and the 2004 Influenza B isolate was diluted in PBS, UTM and M4 at three input levels. Neither of the tests showed a drop in sensitivity with virus diluted in UTM or M4 compared to PBS. Uninoculated UTM, M4 and PBS vials were used as Negative Controls. The Remel devices showed a faint band in both lanes, whether inoculated with Flu A or B, indicated by (+). Low level positive results could not be confirmed because of this problem. M4 caused a strong false Flu A positive band on the Quidel test, indicated by "+ for A", within 10 min. This made diagnosing true Flu A positives impossible, as indicated by the +?. Copan's UTM was compatible with all tests.

FDA Cleared Specimen Collection Types for Each Manufacturer

	Throat swabs	Nasal swabs	Aspirates and NP washes
BD	Yes	No	Yes
Binax	No	Yes	Yes
Quidel	No	Yes	Yes
Remel	Yes	Yes	Yes



POTENTIAL SOLUTIONS:

- Overcoming dual swab collection – placing the swab directly into transport medium (M4 or UTM) would maintain virus viability during transport.
 - Are the POCT devices/methods compatible with this approach?
- Overcoming the instability of nasal aspirates and washes in PBS– placing the aspirate or wash into an approved transport medium (UTM) at an appropriate dilution would enhance the recovery in the laboratory after shipment of the specimen.
 - Does diluting UTM 1:1 with nasal wash aspirate allow for virus recovery substantially equivalent to UTM?

Virus Recovery in UTM and “Diluted” UTM

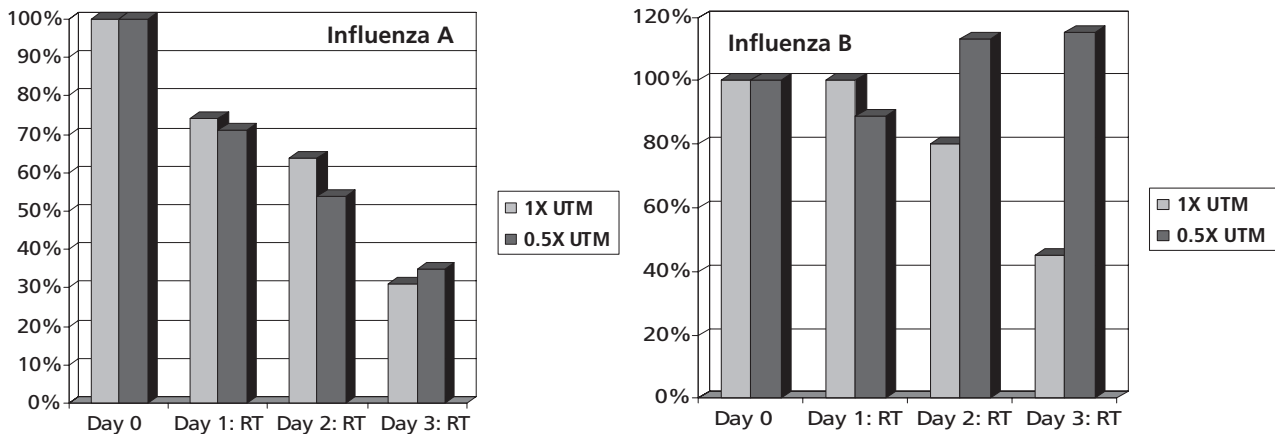


Figure 4: Virus is recovered from vials stored at Room Temperature for up to 72 hours. Not shown, but also tested in 0.5X UTM were RSV, Adenovirus, Para 1, Para 2, Para 3, and 4 sub-types of hMPV. All showed stability was substantially equivalent to 1X UTM.

SUMMARY OF RESULTS

Objective #1: Compare POCT Kits for Analytical Sensitivity

- Detection sensitivity varied intra-kit dependent on virus strain
- Detection sensitivity varied among manufacturers
 - BD's EZ Flu A and B test had the best Flu A sensitivity regardless of virus strain
 - Quidel had the best Flu B sensitivity regardless of virus strain
- As previously shown, R-Mix *Mixed Cells* detected 1 TCID₅₀ of both Influenza A and B, regardless of strain or H/N type

Objective #2: Compatibility of transport media with POCT Kits

- Copan UTM can be used with ALL POCT kits.
- The Quidel POCT is not compatible with the viral transport medium, M4.

Objective #3: Recovery of flu virus in UTM for transport of nasal aspirates and washes

- Flu A, when diluted 1:1 in UTM to simulate a nasal wash or aspirate, shows a similar recovery profile to UTM for 72 hrs
- Flu B, when diluted 1:1 in UTM to simulate a nasal wash or aspirate, shows as good or better recovery profile to UTM for 72 hrs

CONCLUSION

- UTM enables ready compliance using a single specimen (swab, nasal aspirate or wash) for both POCT and reflex testing of “negatives” by R-Mix.