

INTRODUCTION

Improving the detection and characterization of Influenza A is important for both routine influenza diagnostics and pandemic preparedness. A number of in-house and commercial assays are being used in clinical microbiology laboratories. A transport medium and collection system that preserves nucleic acids is essential for use with influenza amplification assays.

OBJECTIVE

To demonstrate the ability of the UTM-RT (Copan Italia) to preserve viral RNA for extraction with different nucleic acids extraction methods and Real-Time PCR assays for the detection of influenza A (FA) and Influenza B (FB).

METHODS

- ❖ Viral transport media (UTM-RT, Copan Italia) was used to generate ten-fold serial dilutions of Influenza A viruses H3N2 and H1N1 and Influenza B cultured viral lysates.
- ❖ Four dilutions of FA and FB viral lysates in viral transport media were chosen to be included in the panel with the lowest dilution being at or near the detection limit.
- ❖ Three replicates for each viral dilution were included for a total of 24 possible FA positives and 12 possible FB positives.
- ❖ Negative samples included hMPV, RSV, and two UTM-RT viral transport media only. The order of samples was randomized; samples were dispensed into 0.5 mL aliquots and panels were sent out on dry ice to 3 different sites.
- ❖ The site that produced the panel also tested it using a blinded technologist.
- ❖ A report sheet was included to provide information on extraction method used, amplification assay(s) used, and criteria for calling a positive.

DESCRIPTION OF ASSAYS

Site	1	2	2	3	4
Extraction Method	bioMérieux miniMag	bioMérieux EasyMag	Qiagen QIAamp mini DNA	Qiagen QIAamp mini DNA	Qiagen RNeasy
Amplification Assay	Conventional RT-PCR	NASBA	Real-time RT-PCR	Real-time RT-PCR	Real-Time RT-PCR
Kit	Invitrogen MMLV Reverse transcriptase	NuclISENS® Basic Kit Amplification Reagents	Qiagen Quantitect RT-PCR probe kit	Qiagen Quantitect RT-PCR probe kit	BioRad iScript one-step RT-PCR probe kit
Reference	Fouchier et al. (2000), unpublished	Moore et al. (2004)	Schweiger et al. (2000), van Elden et al. (2001)	Schweiger et al. (2000), van Elden et al. (2001)	Ward et al. (2004)

RESULTS

One site performed conventional RT-PCR for FA and FB with detection by gel electrophoresis. The remaining sites performed Real-Time RT-PCR assays. Additionally, one of the sites also performed NASBAs for FA and FB on the panel specimens. The conventional RT-PCR detected 23 of 24 (95.8%) FA and 12 of 12 (100%) FB with a specificity of 100%. For FA detection the Real-Time RT-PCR assays had sensitivities of 100% (24/24). For FB detection, sensitivities ranged from 75.0% (9/12) to 91.7% (11/12), missing only those samples at the lowest dilution. One site had one FA false positive. The FA and FB NASBA assays had sensitivities and specificities of 100%.

CONCLUSIONS

- ❖ Influenza A and B samples prepared in the Copan UTM-RT were compatible with the 4 nucleic acid extraction methods and detected by conventional and Real-Time RT-PCR assays and NASBA at a high sensitivity and specificity.
- ❖ The UTM-RT preserved the Influenza A and B RNA even in the samples prepared near the limit of detection.

RESULTS cont'd

#	Identity	Site 1		Site 3		Site 4		Site 2 NASBA		Site 2 Real-Time	
		Matrix FluA	Flu B	FluA	FluB	FluA	FluB	FluA	FluB	FluA	FluB
1	H3-1	+	-	1	0	1	0	1	0	1	0
2	H1-3	+	-	1	0	1	0	1	0	1	0
3	H1-4	+	-	1	0	1	0	1	0	1	0
4	H1-5	-	-	1	0	1	0	1	0	1	0
5	H1-2	+	-	1	0	1	0	1	0	1	0
6	H3-2	+	-	1	0	1	0	1	0	1	0
7	H3-3	+	-	1	0	1	0	1	0	1	0
8	H3-1	+	-	1	0	1	0	1	0	1	0
9	H3-3	+	-	1	0	1	0	1	0	1	0
10	FLUB-3	-	+	0	1	0	1	0	1	0	1
11	FLUB-6	-	+	0	1	0	1	0	1	0	0
12	RSV	-	-	0	0	0	0	0	0	0	0
13	H3-4	+	-	1	0	1	0	1	0	1	0
14	FLUB-4	-	+	0	1	0	1	0	1	0	1
15	FLUB-3	-	+	0	1	0	1	0	1	0	1
16	H3-3	+	-	1	0	1	0	1	0	1	0
17	H1-3	+	-	1	0	1	0	1	0	1	0
18	H1-4	+	-	1	0	1	0	1	0	1	0
19	FLUB-5	-	+	0	1	0	1	0	1	0	1
20	FLUB-6	-	+	0	0	1	0	0	1	0	0
21	H1-2	+	-	1	0	1	0	1	0	1	0
22	H1-4	+	-	1	0	1	0	1	0	1	0
23	H1-5	+	-	1	0	1	0	1	0	1	0
24	FLUB-3	-	+	0	1	0	1	0	1	0	1
25	FLUB-5	-	+	0	1	0	1	0	1	0	1
26	H3-2	+	-	1	0	1	0	1	0	1	0
27	FLUB-4	-	+	0	1	0	1	0	1	0	1
28	NEG	-	-	0	0	0	0	0	0	0	0
29	FLUB-6	-	+	0	0	0	1	0	1	0	0
30	H1-2	+	-	1	0	1	0	1	0	1	0
31	FLUB-4	-	+	0	1	0	1	0	1	0	1
32	hMPV	-	-	0	0	0	0	0	0	0	0
33	H3-4	+	-	1	0	1	0	1	0	1	0
34	NEG	-	-	0	0	0	0	0	0	0	0
35	H1-5	+	-	1	0	1	0	1	0	1	0
36	FLUB-5	-	+	0	1	0	1	0	1	0	1
37	H3-4	+	-	1	0	1	0	1	0	1	0
38	H3-2	+	-	1	0	1	0	1	0	1	0
39	H1-3	+	-	1	0	1	0	1	0	1	0
40	H3-1	+	-	1	0	1	0	1	0	1	0
TOTALS	23 of 24	12 of 12	24 of 24	10 of 12	24 of 24	11 of 12	24 of 24	12 of 12	24 of 24	9 of 12	
Sensitivity Total (A)	97.2%	(95.8%)	94.4%	(100%)	97.2%	(100%)	100%	91.7%	(100%)		
Sensitivity B	100%		83%		91.70%		100%	75%			
Specificity	100%		100%		A-97.5%	(B-96.4%)	100%	100%			