

A FEASIBILITY STUDY OF A NEW NEAR POINT OF CARE INFLUENZA TEST

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Background:

Annually over 200,000 people are hospitalized and 36,000 die in the US from influenza. Diagnosis by clinical manifestations alone is difficult because of overlapping symptoms from a variety of pathogens and respiratory conditions. Standard laboratory analysis by viral culture is resource-intensive and takes days to confirm. Nucleic acid tests (NATs) offer improved sensitivity and specificity but may be limited because of cost, labor time, and diagnosis delay of 8-24 hours. Newer methods offer promise of overcoming these limitations but are untested.

Study Objective:

In a large academic setting, determine the feasibility of a novel near point of care (NPOC) nucleic acid lateral flow (NALF) flu test (Lucigen Corp. Middleton, WI) for prospective observation on a convenience sample of Emergency Department (ED) patients presenting with flu-like symptoms. Outcome measures were sensitivity and specificity.

Methods:

Twenty nasal swab samples were collected from ED patients and eighty confirmed controls obtained from local epidemiology labs were investigated: 24 Flu A H1N1 swine; 12 Flu A H1N1, 12 Flu A H3N2, 15 Flu B seasonal; 5 Adenovirus; 5 *Streptococcus*; 4 Parainfluenza-3; and 3 negative samples. Nucleic acids were extracted with the NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France) following manufacturer's instructions and sample detection for the presence of Flu A or Flu B RNA molecules was completed via PyroScript® amplification. The NALF flu tests employ a unique single enzyme thermostable reverse transcriptase that directly amplifies from Flu A and Flu B RNA. NALF devices use capillary action to bind biotin-labeled amplicons. FAM-labeled probes hybridize to specific amplification products and are visually detected via gold nanoparticle-labeled anti-FAM antibodies. Results can be observed within 2 hours of sample collection.

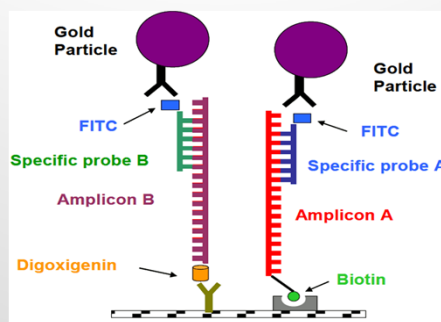


Figure 1. Nucleic Acid Lateral Flow (NALF) Devices. Biotinylated PCR amplicon (green) or labeled digoxigenin amplicon (yellow) enters NALF devices by capillary action bind to streptavidin lines (gray) or anti-digoxigenin Ab (gold). FITC labeled specific probes hybridized to PRC amplicons. FITC (blue) is detected by anti-FITC Abs labeled with colloidal gold particles (purple).

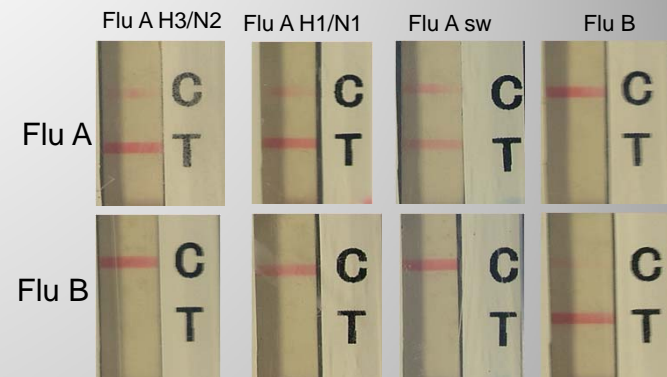


Figure 2. Detection of clinical samples with Influenza A and B NALF Test Cassettes. Control (C), Test (T), and swine (sw).

Results/Conclusions:

20/24 (83.3%), 25/26 (96.2%), and 14/15 (93.3%) were properly identified as Flu A swine, seasonal Flu A, and Flu B samples respectively. 3/20 samples collected from ED patients were detected as two Flu A and one Flu B. The two Flu A ED positive samples were confirmed by the Rapid Flu test. 5/50 and 1/15 positive samples were observed as false negatives resulting in 90% and 93.3% sensitivity for Flu A and Flu B respectively. 3/50 and 6/85 negative samples were found as false positives resulting in 94% and 92.9% specificity for Flu A and Flu B respectively.

Conclusion: The NALF flu tests show promise for rapid NPOC flu screening and warrant further research.

Clinical Samples	comparators				PyroScript®	
	Rapid Flu	X- TAG®	ElectraSense® Influenza A	Strep Culture Pos.	Flu A	Flu B
Flu A swine	n.a.	24	23	n.a.	20/24	2/24
Flu A H1/N1	n.a.	12	12	n.a.	12/12	2/12
Flu A H3/N2	n.a.	12	12	n.a.	11/12	0/12
Flu B	n.a.	15	n.a.	n.a.	1/15	14/15
ED Samples	2/20	n.a.	n.a.	n.a.	2/20	1/20
<i>Streptococcus</i>	n.a.	n.a.	n.a.	5	0/5	0/5
Adenovirus	n.a.	5	n.a.	n.a.	0/5	0/5
Parainfluenza 3	n.a.	4	n.a.	n.a.	1/4	1/4
Negative	n.a.	3	n.a.	n.a.	1/3	0/3

Table 1. Results of PyroScript® Influenza A and B assays compared against results from Rapid Flu, X- TAG®, ElectraSense® Influenza A, and Strep Culture tests. Gray fill indicates comparator assay not applicable (n. a.) and therefore not performed.