PERFORMANCE COMPARISON OF COMMERCIAL MOLECULAR ASSAYS TO DETECT AND IDENTIFY PANDEMIC INFLUENZA A IN A SENSITIVITY PANEL AND IN CLINICAL SPECIMENS

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Revised Abstract

Objective: The objective of this study was to compare the sensitivity and specificity of commercially available molecular screening and subtyping assays to detect seasonal and pandemic 2009 H1N1 influenza A.

Methods: A panel of 30 samples containing serial dilutions of viral lysate from 3 influenza A subtypes (pandemic 2009 H1N1, seasonal H1N1 and H3N2) and 5 influenza A negative samples was tested with 4 commercial influenza A screening and subtyping assays. The panel was designed to challenge the lower limit of detection in the 4 assays; the Influenza Screen & Type RT-PCR Kit (astra Diagnostics), Influenza A/H1N1 Detection Set (Roche), Luminex x-Tag™ RVP fast and ProFLU+™ /ProFLU™-ST assays (Prodesse) were tested according to manufacturer's instructions. The performance of these assays was further investigated using 100 NPS samples which included 51 influenza A negatives and 10 seasonal H1N1, 10 seasonal H3N2 and 29 pandemic H1N1 positive samples as previously determined by an influenza A matrix PCR. The sensitivity and specificity of each assay was determined using a combined reference standard of positivity in at least 2 assays.

Results: The commercial assays were able to detect 27 to 60 % of the 15 potential pandemic H1N1 positives and 50 to 80 % of the 10 potential seasonal flu positives in the panel. For the clinical samples, the commercial assays had a sensitivity and specificity for influenza A screening ranging from 82 to 100%. The pandemic H1N1 subtyping assays had a sensitivity and specificity ranging from 92 to 100%. With the clinical samples, only the Influenza Screen and Type RT-PCR assay from astra Diagnostics allowed accurate simultaneous identification of influenza A and 2009 H1N1 (sensitivity 100% specificity 100%).

Conclusions: The performance of commercial assays for influenza A screening, particularly for pandemic H1N1, was variable. Proper validation of the screening assay is therefore critical for the identification of pandemic H1N1.

Objective

The objective of this study was to compare the sensitivity and specificity of commercially available molecular screening and subtyping assays to detect seasonal and pandemic 2009 H1N1 influenza A.

Methods

Commercial assays:

- Influenza Screen & Type RT-PCR Kit (astra Diagnostics)
- Influenza A/H1N1 Detection Set (Roche):Ver 1 (05/ 2009), Ver 2 (08/2009)
- x-Tag™ RVP fast (Luminex)
- ProFLU™+ /ProFLU™-ST assays (Prodesse)

Assays were tested according to manufacturer's instructions

Sensitivity panel:

Panel of extracted nucleic acid from 30 randomized samples consisting of viral lysate dilutions of a pandemic isolate (A/swine/California/04/2009-like), a seasonal H3N2 strain (A/Victoria/3/75), a seasonal H1N1 strain (A/Solomon Islands/03/06) and 5 influenza A negative samples (Flu B, hMPV, Para 3 and 2 water blanks)

Clinical Evaluation:

- 100 NPS samples (Copan FLOQSwabs™) collected in UTM-RT (Copan): 49 influenza A positives (29 pandemic H1N1, 10 seasonal H1N1, 10 seasonal H3N2) and 51 influenza A negative samples as previously determined by an influenza A matrix PCR
- Purified nucleic acid from 200 uL of NPS sample extracted by easyMAG (bioMérieux), eluted in 60 uL and tested in the 4 commercial assays
- The sensitivity and specificity of each assay was determined using a combined reference standard of positivity in at least 2 assays

Results of Sensitivity Panel

Sensitivity Panel			astra Diagnostics	Roche Diagnostics	Luminex	Prodesse
#	Panel Contents	Panel #	Influenza A Screen & Type RT-PCR	2009 H1N1 Detection Set Ver 1	x-Tag™ RVP fast	ProFlu™ plus & ProFlu™-ST
1	2009 H1N1 Dilution 1-1	15	2009 H1N1	2009 H1N1	Flu A	2009 H1N1
2	2009 H1N1 Dilution 1-2	28	2009 H1N1	Flu A	Flu A	2009 H1N1
3	2009 H1N1 Dilution 1-3	24	2009 H1N1	2009 H1N1	Flu A	2009 H1N1
4	2009 H1N1 Dilution 2-1	7	2009 H1N1	2009 H1N1	Flu A	2009 H1N1
5	2009 H1N1 Dilution 2-2	23	2009 H1N1	2009 H1N1	Flu A	Flu A; untypeable
6	2009 H1N1 Dilution 2-3	30	2009 H1N1	Flu A	Flu A	2009 H1N1
7	2009 H1N1 Dilution 3-1	10	2009 H1N1	Neg	Negative	Flu A; untypeable
8	2009 H1N1Dilution 3-2	27	2009 H1N1	Neg	Flu A	Flu A; untypeable
9	2009 H1N1 Dilution 3-3	16	2009 H1N1	Neg	Flu A equiv	Neg
10	2009 H1N1 Dilution 4-1	20	Neg	Neg	Negative	Flu A; untypeable
11	2009 H1N1 Dilution 4-2	11	Neg	Neg	Negative	Neg
12	2009 H1N1 Dilution 4-3	3	Neg	Neg	Negative	Neg
13	2009 H1N1 Dilution 5-1	22	Neg	Neg	Negative	Neg
14	2009 H1N1 Dilution 5-2	5	Neg	Neg	Negative	Neg
15	2009 H1N1 Dilution 5-3	19	Neg	Neg	Negative	Neg
16	H1N1 Seasonal Dilution 1	18	Flu A	Flu A	Flu A, H1	Flu A; H1
17	H1N1 Seasonal Dilution 2	4	Flu A	Flu A	Flu A, H1	Flu A; H1
18	H1N1 Seasonal Dilution 3-1	1	Neg	Flu A	Negative	Flu A; H1
19	H1N1 Seasonal Dilution 3-2	14	Neg	Neg	Negative	Flu A; untypeable
20	H1N1 Seasonal Dilution 3-3	6	Flu A	Neg	Negative	Flu A; H1
21	H3N2 Seasonal Dilution 1	29	Flu A	Flu A	Flu A, H3	Flu A; H3
22	H3N2 Seasonal Dilution 2	26	Flu A	Flu A	Flu A, H3	Flu A; untypeable
23	H3N2 Seasonal Dilution 3-1	13	Flu A	Neg	Flu A, H3	Flu A; untypeable
24	H3N2 Seasonal Dilution 3-2	8	Flu A	Neg	Flu A, H3	Flu A; untypeable
25	H3N2 Seasonal Dilution 3-3	17	Flu A	Neg	Flu A, H3 equiv	Flu A; untypeable
26	dH2O	21	Neg	Neg	Negative	Neg
27	dH2O	25	Neg	Neg	Negative	Neg
28	Flu B	2	Neg	Neg	Flu B	Flu B
29	hMPV	9	Neg	Neg	MPV	Neg
30	Para 3	12	Neg	Neg	Para 3	Neg

Panel Sensitivity for 2009 H1N1

astra Diagnositics
- 9/15 = 60.0%

Roche Diagnostics
- 6/15 = 26.7 %; 2 untypeable influenza A +ve

Luminex
- 7/15 = 46.7 %; 1 equivocal influenza A +ve

Prodesse
- 5/15 = 33.3 %; 4 untypeable influenza A +ve

Panel Sensitivity for Seasonal Influenza

astra Diagnositics
- 8/10 = 80.0%; not typed

Roche Diagnostics
- 5/10= 50.0%; not typed

Luminex
- 6/10 = 60.0 %; 1 equivocal
- H1, 2/5 = 40.0%; H3, 4/5 = 80.0%

Prodesse
- 5/10 = 50.0%; 4 untypeable
- H1, 4/5 = 80.0%; H3, 1/5 = 20.0%

Results of Clinical Evaluation

Sample Breakdown	astra Diagnostics	Roche Diagnostics Ver 1		Roche Diagnostics Ver 2		Luminex	Prodesse	
	Screen & Type	Inf A/M2	Inf A/H1 (swine)	Inf A/M2	Inf A/H1 (swine)	x-Tag™ RVP fast	ProFLU™ plus	ProFLU™ ST
29 2009 H1N1+	29/29 2009 H1N1+	13/17 Flu A+	17/17 2009 H1N1+	12/12 Flu A+	12/12 2009 H1N1+	26/29 Flu A+	28/29 Flu A+	25/29 2009 H1N1+
10 H1N1+	20/20 Flu A+	10/11 Flu A+	11/11 2009 H1N1-	9/9 Flu A+	9/9 2009 H1N1-	10/10 H1+	20/20 Flu A+	10/10 H1+
10 H3N2+						10/10 H3+		10/10 H3+
51 Flu A-	51/51 Flu A-	22/22 Flu A-	22/22 2009 H1N1-	27/29 Flu A-	29/29 2009 H1N1-	51/51 Flu A-	51/51 Flu A-	51/51 Flu A-
100 Total	100 Tested	50 Tested	50 Tested	50 Tested	50 Tested	100 Tested	100 Tested	100 Tested

- The 51 influenza A negative samples included 11 samples positive for other respiratory viruses: 1 adenovirus, 3 RSV- A, 4 enterovirus/rhinovirus, 1 influenza B, 1 para 1 and 1 para 4

Clinical Performance

astra Diagnostics Influenza Screen & Type RT-PCR Kit:
- no false negatives or false positives

Roche Diagnostics Influenza A/H1N1 Detection Set:
- InfA/M2 assay:
  - Ver 1, 4 pandemic H1N1 false negatives
  - Ver 1, 1 seasonal H1N1 false negative
  - Ver 2, 2 false positive influenza A negative samples
  - InfA/H1 (swine) assay: no false negatives or false positives

Luminex x-Tag™ RVP fast:
- 3 pandemic H1N1 false negatives

Prodesse ProFLU™+ assay:
- 1 pandemic H1N1 false negative

Prodesse ProFLU-ST assay:
- 4 pandemic H1N1 false negatives

Clinical Sensitivity and Specificity

	astra Screen & Type	Roche inf A/M2 Ver 1	Roche inf A/M2 Ver 2	Roche inf A/H1 Ver 1	Roche inf A/H1 Ver 2	Luminex x-Tag™ RVP fast	Prodesse ProFLU™ +	Prodesse ProFLU™ ST
Sensitivity	49/49 (100%)	23/28 (82.1%)	21/21 (100%)	17/17 (100%)	12/12 (100%)	46/49 (93.9%)	48/49 (98.0%)	45/49 (91.8%)
Specificity	51/51 (100%)	22/22 (100%)	27/29 (93.1%)	33/33 (100%)	38/38 (100%)	51/51 (100%)	51/51 (100%)	51/51 (100%)
PPV	49/49 (100%)	23/23 (100%)	21/23 (91.3%)	17/17 (100%)	12/12 (100%)	46/46 (100%)	48/48 (100%)	45/45 (100%)
NPV	51/51 (100%)	22/27 (81.5%)	27/27 (100%)	33/33 (100%)	38/38 (100%)	51/54 (94.4%)	51/52 (98.1%)	51/55 (92.7%)

Conclusions

- The sensitivity of the commercial assays for influenza A screening, particularly for pandemic H1N1, was variable (82.1-100%)
- Proper validation of an influenza A screening assay is critical to the identification of 2009 H1N1
- Only the Influenza Screen and Type RT-PCR assay from astra Diagnostics allowed simultaneous identification of influenza A and 2009 H1N1 with a 100% sensitivity and specificity, in clinical samples