

Significant Increase of Sensitivity on Rapid Influenza Antigen Assays Using Silver Amplification Immunochromatography

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Aim and Background:

- Influenza virus has been recognized as one of the most pandemic infectious disease agent. Rapid diagnosis of influenza is commonly performed with immuno-chromatography method (IC) using specimens taken from upper respiratory tract.
- Although IC is easy and relatively low price, its sensitivity is not perfect especially in early stage of disease because of low concentration of virus antigens¹⁾.
- In order to increase sensitivity, we developed a new IC method applying "silver amplification (SI)" principle²⁾, ³⁾. **Fig. 1**
- **The aim of this study** is to clarify if our method has higher sensitivity and specificity than conventional IC assays.

Materials and Methods:

- One hundred and twenty cases of influenza-like symptoms; fever, rhinorrhea, cough, and/or general fatigue who visited pediatrics department of our hospital from November 2011 to April 2012 were entitled.
- Cotton swab specimens were applied to Fuji Drychem IMMUNO AG1™ (FUJIFILM Medical, Tokyo, Japan). **Fig. 2**
- Simultaneously, a conventional IC method was performed with Quick Chaser FLU A/ B™ by Mizuho Medy (Tosu, Japan).
- A real time PCR with TaqMan probe was also done for gold standard.
- This study was authorized by local ethic committee and written informed consent was obtained from all the cases.

Results:

- With SI method, 22, 15, 1 cases were influenza A, B, and both positive, respectively. **Tab. 1**
- On the other hand, with IC method, 23, 8, 0 cases were A, B, and both positive. **Tab. 1**
- All the cases, which showed positive in SI, negative in IC for influenza B assays, PCR results were positive. **Tab. 2 and 3**
- Their virus concentration ranged 10⁵ to 10⁹ copies/ml. **Tab. 4**
- Although sensitivity of SI for influenza B assay was higher than that of IC, it was not statistically significant (p=0.085).

Fig.1: Mechanism of Silver Amplification Method

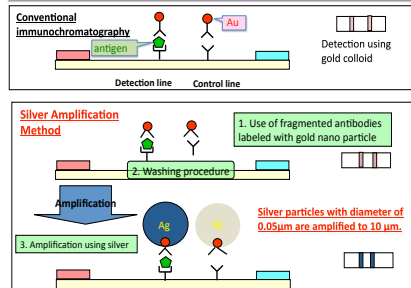
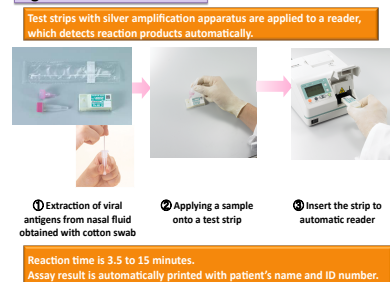


Fig.2: ASSAY PROCEDURE



Tab.1: Comparison of silver amplification method and conventional immunochromatography (N=120)

Immunochromatography	silver amplification	→	AB	negative	cases
A	22	0	1	0	23
B	0	8	0	0	8
AB (both)	0	0	0	0	0
negative	0	7	0	82	89
total	22	15	1	82	120

Seven cases were positive with silver amplification although negative with immunochromatography.

Tab.2: Comparison of silver amplification method and real time PCR (N=120)

silver amplification	RT-PCR	→	AB	negative	total
A	20	0	0	2	22
B	0	15	0	0	15
AB (both)	1	0	0	0	1
negative	0	0	0	82	82
total	21	15	0	84	120

silver amplification	%	cases
sensitivity (A)	100	21/21
sensitivity (B)	100	15/15
specificity	97.6	82/84

Tab.3: Comparison of conventional immunochromatography and real time PCR (N=120)

Immunochromatography	RT-PCR	→	AB	negative	cases
A	21	0	0	2	23
B	0	8	0	0	8
AB (both)	0	0	0	0	0
negative	0	7	0	82	89
total	21	15	0	84	120

immunochromatography	%	cases
sensitivity (A)	100	21/21
sensitivity (B)	53.3	8/15
specificity	97.6	82/84

Discussion:

- Specificity and sensitivity for influenza A was not different between the two assays. However, SI showed higher sensitivity for influenza B.
- Because SI applies photo-developing principle, its sensitivity was reported to increase 8 to 16 times⁴⁾. Hara et al⁵⁾ reported this method has 32 times higher sensitivity especially influenza B.
- Hikita⁶⁾ reported SI detects influenza B antigen significantly earlier than IC. The average hour from onset to show positive result was 3 hours with SI, 15 hours with IC.
- Because this automatic reader is small (18x20x11cm) and light (1.8kg), it is suitable for bedside testing especially in emergency room. Reproducibility among examiners are excellent because it does not require human visual detection.
- Since number of cases is limited, more data are required to confirm the result.

Tab.4: Profiles of discrepancy cases

Case	Immunochromatography	Silver amplification	RT-PCR (copies/ml)
M	(-)	B(+)	B(+): 1.47E+6
M	A(+)	A(+): B(+)	A(+): 4.09E+5, B(+)
M	(-)	B(+)	B(+): 5.11E+5
M	(-)	B(+)	B(+): 4.97E+5
M	(-)	B(+)	B(+): 3.20E+5
M	(-)	B(+)	B(+): 7.08E+04
M	(-)	B(+)	ND
F	(-)	B(+)	ND

ND: not done

Conclusion:

Our novel assay method using silver amplification has high potentiality to increase sensitivity of rapid bedside testing diagnosis especially for influenza B.

References:

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