Electronic Supplementary Information (ESI)

Pre-clinically evaluated visual lateral flow platform using influenza A and B nucleoprotein as a model and its potential applications

Natpapas Wiriyachaiporn*[1], Siriwan Sirikaew[1], Nawakarn Chitchai[2], Pareena Janchompoo[3], Weerakanya Maneeprakorn[1], Suwussa Bamrungsap[1], Ekawat Pasomsub[3], Deanpen Japrung[1]

[1]National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA), PathumThani, 12120, Thailand

[2]Faculty of Pharmacy, Thammasat University

[3]Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

*Corresponding author

Phone: +66 2117 6500

Fax: +66 2564 6981

Email address: natpapas@nanotec.or.th

Supplementary Figures

Fig. S1 Expression of virus proteins on electrophoresis using **(a)** 15% SDS-PAGE and **(b)** Western blot analysis with the dilution of mAb specific to influenza A. Characterization of mAb specific to influenza B using **(c)** SDS-PAGE and **(d)** Western blot analysis was also shown. Lane M: Ladder; Lane 1: Influenza A recombinant nucleoprotein; Lane 2: Inactivated Influenza B infected cell lysate protein; Lane 3: Inactivated RSV infected cell lysate protein; Lane 4: Inactivated adenovirus infected cell lysate protein; Lane 5: Inactivated parainfluenza infected cell lysate protein. Arrow indicates the target protein which is detected by mAb specific to influenza A and B nucleoprotein, respectively.

Fig. S2 Dynamic light scattering data for the size distributions of (top) GNP before and after conjugation to mAbs specific to (middle) influenza A and (bottom) B virus. Red, green, and blue color lines can be referred to as repeat number 1, 2, and 3, respectively.

Fig. S3 Absorption spectra and color of GNP upon the addition of high salt concentration to the systems. These include plain GNP without antibody conjugation (1) before and (4) after adding high salt concentration, (2) GNP-mAb Flu A, and (3) GNP-mAb Flu B.

Fig. S4 Performance evaluation of the commercial tests, designated as commercial test 1 and 2, respectively using serial dilutions of influenza A antigens, compared to the performance of the system developed in this work. Highlighted color indicates the LOD of the systems.

Fig. S5 Performance evaluation of the commercial tests, designated as commercial test 1 and 2, respectively using serial dilutions of influenza B antigens, compared to the performance of the system developed in this work. Highlighted color indicates the LOD of the system.

Fig. S6 Correlation test results of the system for (a) influenza A and (b) B viruses, compared to commercial rapid test and molecular approach. Calculation of percent sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy was obtained.

Fig. S7 Calculation of parameter used in clinical evaluation. True positive and true negative samples indicate those sample correctly determined to be positive and negative, respectively, matched with the results obtained from reference methods. False positive and false negative samples indicate those samples incorrectly determined to be positive and negative, respectively, as compared with the results obtained from reference methods. All diseased patients can be referred to as patients who have influenza, whilst non-diseased patients can be referred to as patients who do not influenza, as identified by reference methods.

Fig. S1

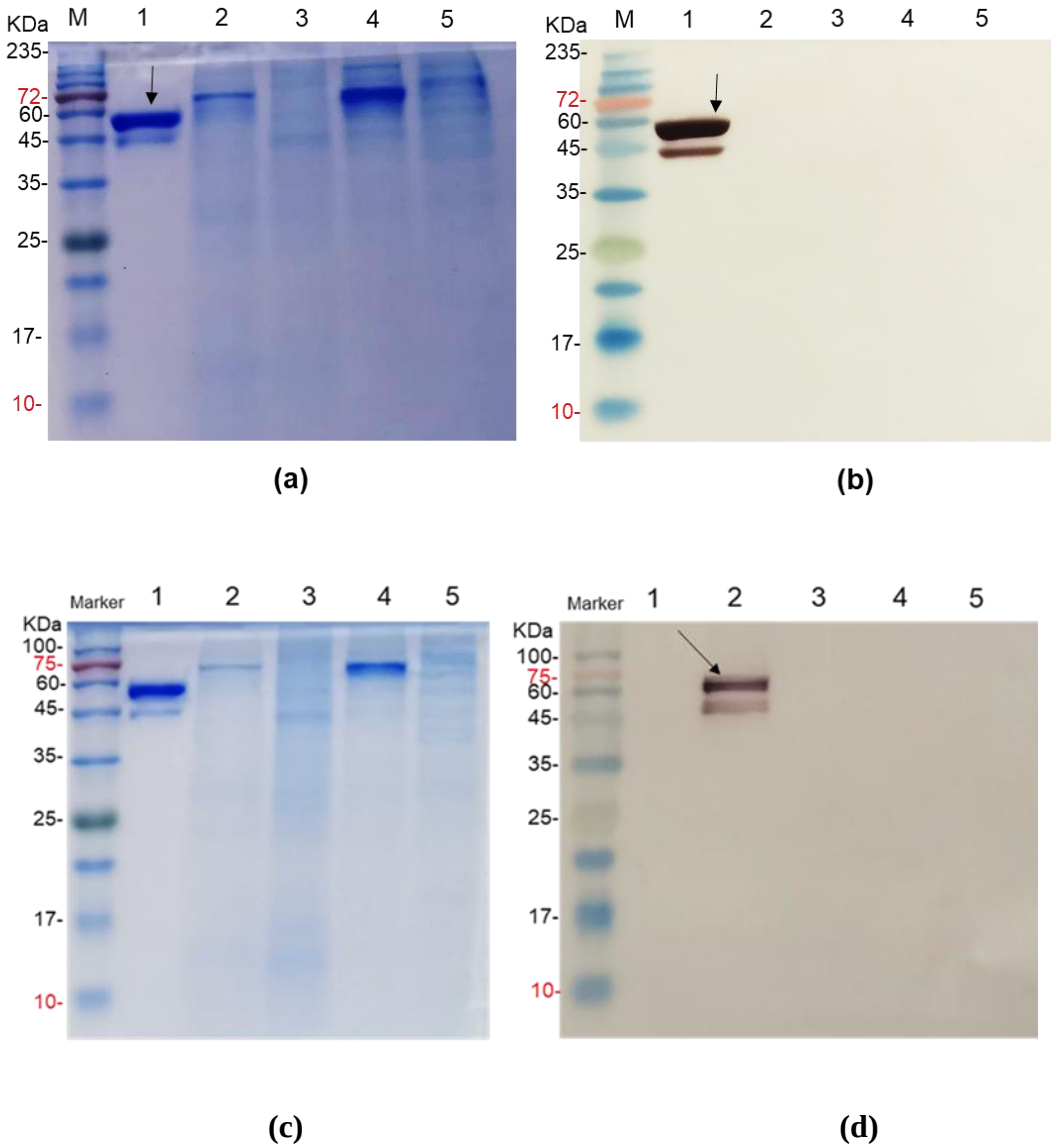

KDa
M
1
2
3
4
5
235-
72-
60-
45-
35-
25-
17-
10-
(a)
KDa
M
1
2
3
4
5
235-
72-
60-
45-
35-
25-
17-
10-
(b)
Marker
1
2
3
4
5
KDa
100-
75-
60-
45-
35-
25-
17-
10-
(c)
Marker
1
2
3
4
5
KDa
100-
75-
60-
45-
35-
25-
17-
10-
(d)

Fig. S2

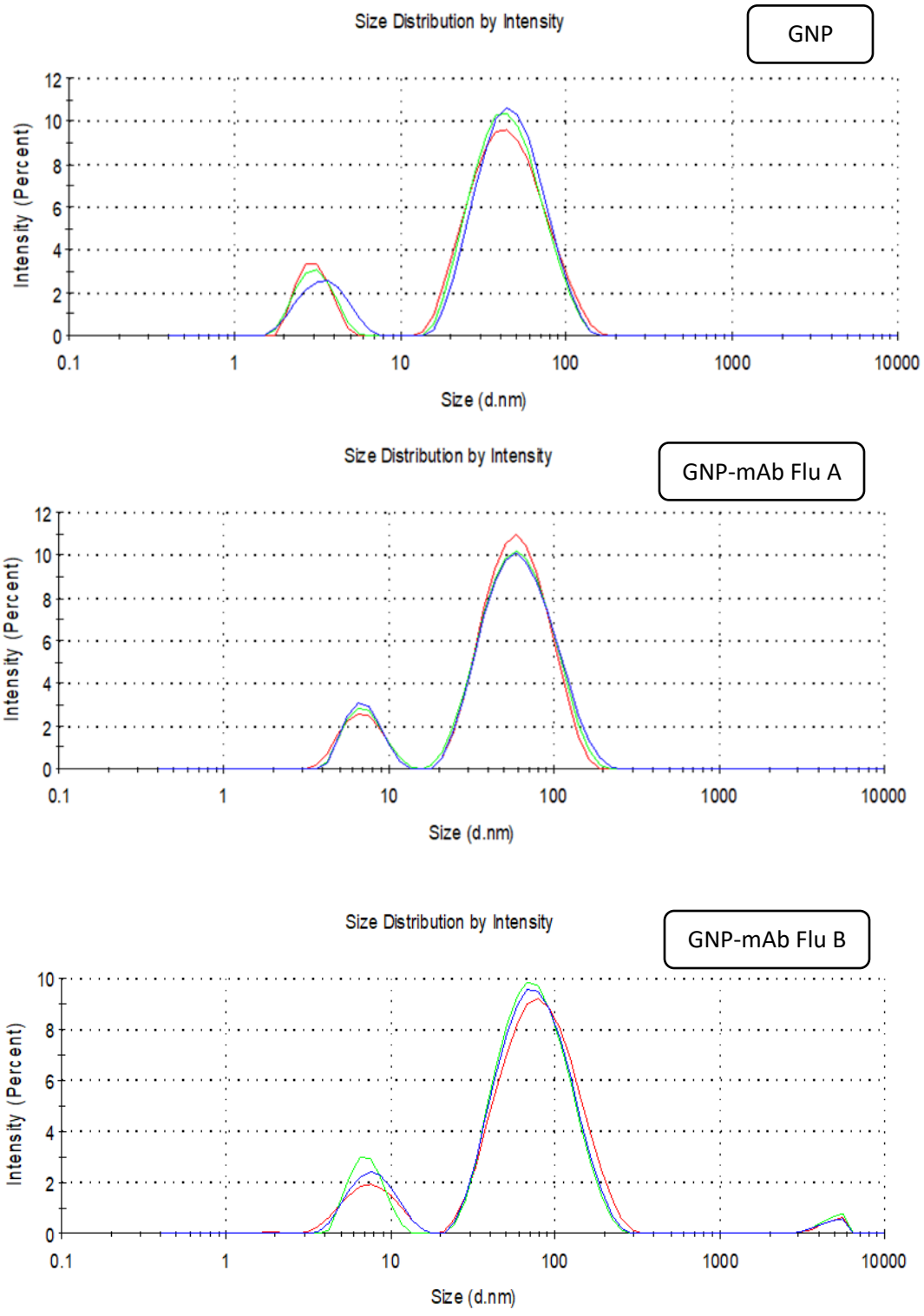

Size Distribution by Intensity
GNP
Intensity (Percent)
Size (d.nm)
Size Distribution by Intensity
GNP-mAb Flu A
Intensity (Percent)
Size (d.nm)
Size Distribution by Intensity
GNP-mAb Flu B
Intensity (Percent)
Size (d.nm)

Fig. S3

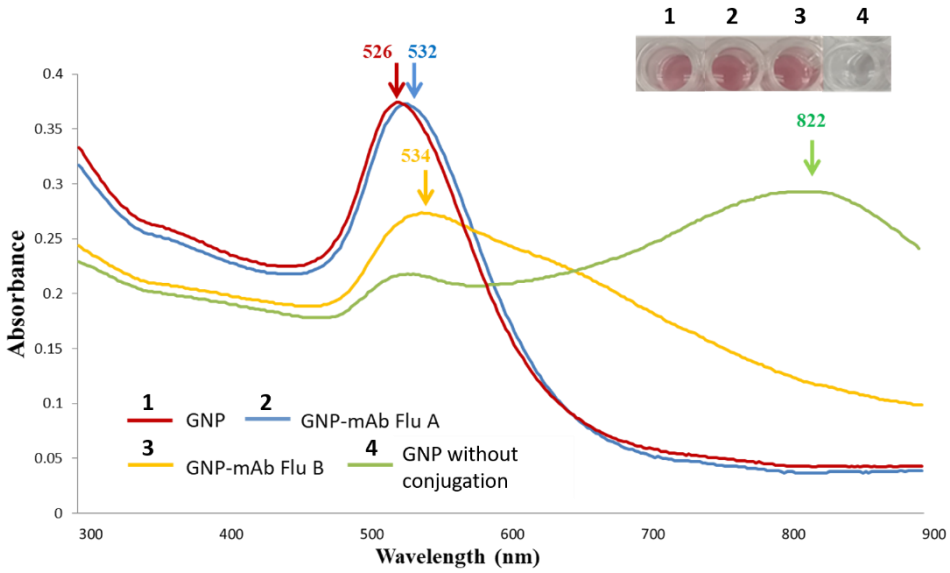

1
2
3
4
526
532
534
822
Absorbance
Wavelength (nm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
300
400
500
600
700
800
900
1 GNP
2 GNP-mAb Flu A
3 GNP-mAb Flu B
4 GNP without conjugation

Fig. S4

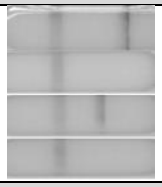
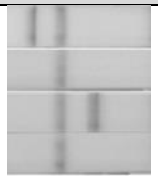
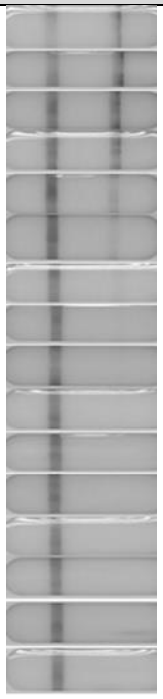
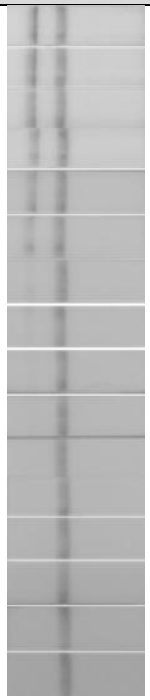
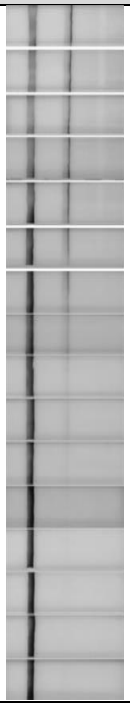
No	Sample (protein antigen/cell lysate protein)	Result at TL1 position (Flu A)					
		Commercial Test				This work	
		1		2			
		Signal C B A	Flu A signal	Signal A C B	Flu A signal	Signal C A B	Flu A signal
1	Influenza A		+		+		+
2	Negative control (buffer only)		-		-		-
3	Influenza B		-		-		-
4	RSV		-		-		-
No	**Influenza A protein antigen (ng/test)**	**Signal C B A**	**Flu A signal**	**Signal A C B**	**Flu A signal**	**Signal C A B**	**Flu A signal**
1	300		+		+		+
2	150		+		+		+
3	75		+		+		+
4	37.5		+		+		+
5	18.8		+		+		+
6	9.4		+		+		+
7	4.8		+		+		+
8	2.4		+		+		+
9	1.2		+		+		+
10	0.6		+		+		+
11	0.3		+		+		+
12	0.15		+		-		+
13	0.08		-		-		+
14	0.04		-		-		+
15	0.02		-		-		-
16	0.01		-		-		-

Fig. S5

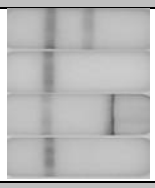
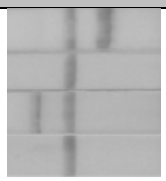
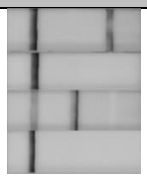
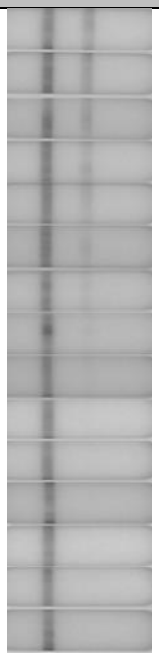
No.	Sample (protein antigen/cell lysate protein)	Result at TL2 position (Flu B)					
		Commercial Test				This work	
		1		2			
		Signal C B A	Flu B Signal	Signal A C B	Flu B Signal	Signal C A B	Flu B Signal
1	Influenza B		+		+		+
2	Negative control (buffer only)		-		-		-
3	Influenza A		-		-		-
4	RSV		-		-		-
No.	**Influenza B protein antigen (ng/test)**	**Signal C B A**	**Flu B Signal**	**Signal A C B**	**Flu B Signal**	**Signal C A B**	**Flu B Signal**
1	8,750		+		+		+
2	4,375		+		+		+
3	2,188		+		+		+
4	1,094		+		+		+
5	547		+		+		+
6	274		+		+		+
7	137		+		+		+
8	68		+		+		+
9	34		+		+		+
10	16		+		+		+
11	8		+		+		+
12	4		+		+		+
13	2		-		-		+
14	1		-		-		+
15	0.5		-		-		-

Fig. S6

(a) Influenza A

This work	Reference test		Total
	Positive	Negative	
Positive	40	-	40
Negative	5	44	49
Total	45	44	89

Sensitivity (%)	40/(40+5) x 100 = 88.8%
Specificity (%)	44/(44+0) x 100 = 100%
PPV (%)	40/(40+0) x 100 = 100%
NPV (%)	44/(5+44) x 100 = 89.8%
Accuracy (%)	40+44/(40+44+0+5) x 100 = 94.4%

(b) Influenza B

This work	Reference test		Total
	Positive	Negative	
Positive	10	-	10
Negative	2	44	46
Total	12	44	56

Sensitivity (%)	10/(10+2) x 100 = 83.3%
Specificity (%)	44/(44+0) x 100 = 100%
PPV (%)	10/(10+0) x 100 = 100%
NPV (%)	44/(2+44) x 100 = 95.7%
Accuracy (%)	10+44/(10+44+0+2) x 100 = 96.4%

Fig. S7

		Reference test		
		Positive	**Negative**	
This work	**Positive**	TP	FP	Test positive results
	Negative	FN	TN	Test negative results
		All diseased patients	Non-diseased patients	Total

TP = True Positive, TN = True Negative, FP = False Positive, FN = False Negative

Sensitivity (%)	TP/(TP+FN) x 100
Specificity (%)	TN/(TN+FP) x 100
PPV (%)	TP/(TP+FP) x 100
NPV (%)	TN/(FN+TN) x 100
Accuracy (%)	TP+TN/(TP+TN+FP+FN) x 100