Design of influenza diagnostic instrument based on biochemiluminescence

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Abstract. The design principle of a biochemiluminescent instrument is presented. The instrument diagnoses influenza based on detection of the flu viral neuraminidase activity. The instrument uses a photomultiplier tube (PMT) as sensor, with 24 test channels. The reaction disk is driven by step motor to rotate, where the reagent is incubated. Finally, PMT detects the number of photons emitted by the reagent and calculates the enzyme activity. The correlation coefficient (R^2) of 2.83 to 5.06 log $TCID_{50}$/ml influenza virus was 0.9967; the detection limits of the two positive virus samples were $TCID_{50}$/mL and 953 $TCID_{50}$/mL, respectively, The positive predictive value (PPV) was 100%, and the negative predictive value (NPV) was 98.90% in clinical evaluation. According to the evaluation, the designed biochemiluminescent instrument can well match with the reagents, and meet with the performance requirements of clinical setting.

1 INTRODUCTION

Influenza is caused by influenza virus infection. Influenza A, B, and C are 3 types of viruses responsible for illness, and type A is most likely to cause epidemic [1]. Influenza is mainly transmitted by air droplets. It often causes chills, headache, persistent cough, fatigue, nasal congestion and sore throat. In severe cases, it can cause pneumonia, myocarditis and heart failure.

The benefit of treatment is greatest when antiviral therapy is started within 24 hours of symptom onset, so a reliable diagnostic method enabled rapid test of influenza is essential [2][3]. At present, polymerase chain reaction (PCR) and colloidal gold are two main methods for influenza detection. PCR assay is based on the detection of flu viral gene sequences and colloidal gold is based on antigens, which are susceptible to genetic change of the viruses. In fact, new influenza viruses are constantly evolving by mutation or by reassortment, so a new detection method is needed for rapid diagnosis [4].

Neuraminidase is one of the essential enzymes of influenza virus, which help progeny influenza virus cleave sialic acid and release from the host cell. Neuraminidase also contributes to virus binding to the sialic acid groups of cell glycoproteins, which could complement the receptor-binding function of hemagglutinin, enhancing enzymatic activities of neuraminidase, and facilitate virus infection [2]. Therefore, neuraminidase plays an important role in virus replication and transmission [5][6]. All type A and B influenza viruses carry neuraminidase, so neuraminidase can be an ideal marker for diagnosis of influenza [7][8]. An assay method, the homogeneous biochemiluminescent assay (HBA) for rapid detection of influenza by detecting viral neuraminidase activity, has been developed [9][10]. It uses a luciferase-based biochemiluminescent substrate [11]. In the presence of influenza virus in a reaction, the substrate is cleaved to free luciferin, which becomes an active substrate of firefly luciferase. In the presence of firefly luciferase, the free luciferase is oxidized to oxyluciferin, resulting in a stable light signal that can be detected by instrument [12][13]. In this paper, we introduce the implementation of a HBA instrument for rapid influenza test based on viral neuraminidase activity.

2 METHODOLOGY

The whole instrument consists of reaction disk, heating module, measuring module, moving and supporting structure, as shown in Figure 1. The reaction disk has 24 test channels, which is the place for reaction mix incubation, reaction and luminescence. The reaction disk is fixed on a step motor. The motor moves to bring the reaction mix to the detection position, and the PMT is directly below the detection position, which is used to measure the light signal emitted by the reagent. The heating module is located at the inner side of the reaction disk, and pasted with a heating film and a temperature sensor. When the instrument works, the heating film and the temperature sensor works together to provide a constant temperature environment for the reaction disk. The instrument is equipped with a shield to prevent stray

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light from entering into the instrument and improve the performance of the instrument.

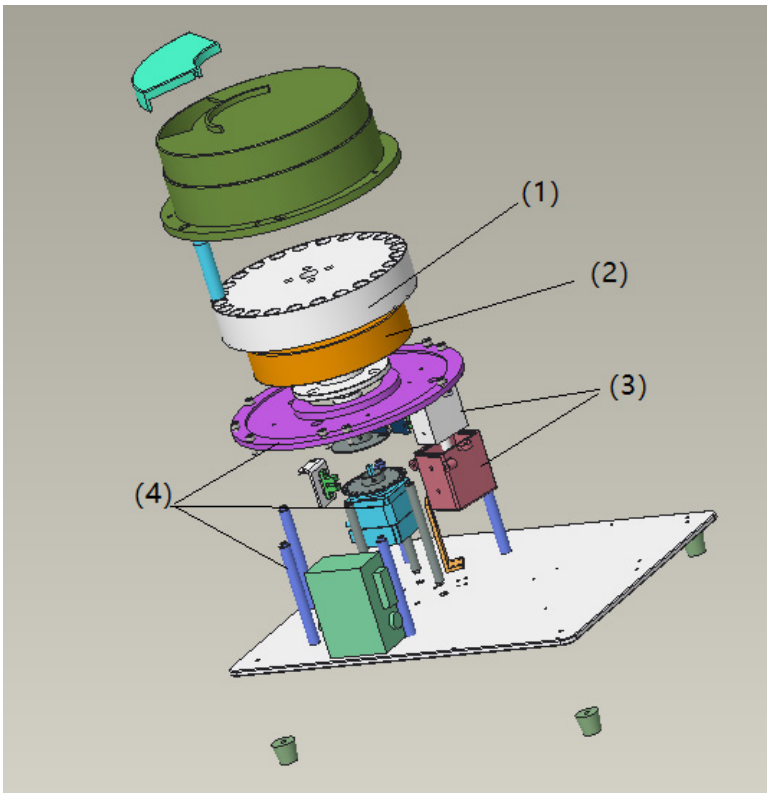

Fig.1. Instrument structure diagram. (1) Reaction disk; (2) heating module; (3) measuring module; (4) moving and supporting structure.

The results of the instrument are read as Relative Light Unit(RLU).A study was conducted to calculate the appropriate cut-off value. We tested 460 clinical samples, and the samples were cultured for virus as well. The RLU results and virus culture results are listed in the table1. With the raw data, the true positive rate (TPR) and false positive rate (FPR) were calculated under different cut-off values.

Table1. The TPR and FPR under different cut-off value

Cut-off (k RLU)	Cultured / Test	P / P	P / N	N / P	N / N	TPR (%)	FPR (%)
50		39	14	152	255	73.58	37.35
100		37	16	79	328	69.81	19.41
180		36	17	46	361	67.92	11.30
190		35	18	43	364	66.04	10.57
200		35	18	36	371	66.04	8.85
210		35	18	35	372	66.04	8.60
220		35	18	32	375	66.04	7.86
230		34	19	31	376	64.15	7.62
240		34	19	28	379	64.15	6.88
300		33	20	14	393	62.26	3.44
400		33	20	9	398	62.26	2.21
500		31	22	3	404	58.49	0.74
600		31	22	0	407	58.49	0.00
700		30	23	0	407	56.60	0.00
800		29	24	0	407	54.72	0.00
900		28	25	0	407	52.83	0.00
1,000		26	27	0	407	49.06	0.00
1,100		26	27	0	407	49.06	0.00
1,200		26	27	0	407	49.06	0.00
1,300		26	27	0	407	49.06	0.00

Cut-off (k RLU)	Cultured Test	P P	P N	N P	N N	TPR (%)	FPR (%)
1,400		26	27	0	407	49.06	0.00
1,500		25	28	0	407	47.17	0.00

460 clinical samples were tested. TPR, FPR were calculated under different cut-off value. Gold standard method was virus culture. P: Positive; N: Negative. P: Positive; N: Negative.

The ROC curve is shown in Figure 2. Since the instrument focuses on clinical screening, in this study, 220K (220000 RLU) was used as the cut-off value. The TPR, FPR were 66.04%, 7.86%, respectively.

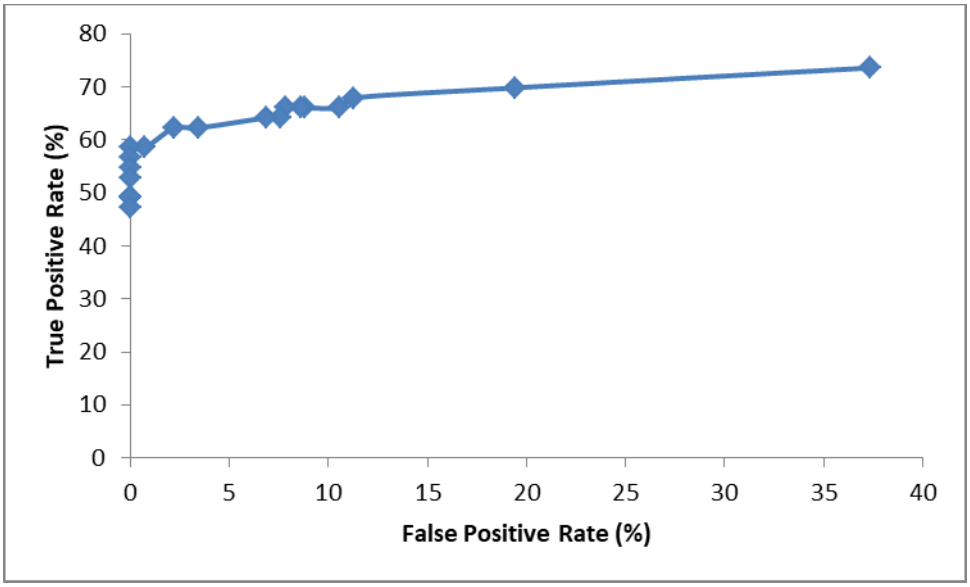

Fig. 2. The ROC curve of 460 clinical samples. Cut-off value ranges from 50K to 1500K RLU. TPR (%) was plotted against FPR (%).

3 RESULTS

To evaluate linearity and linear range, we tested samples of influenza virus strains (A/CA/07/2009; wild type) with concentrations of 2.83 to 5.06 log TCID50 /ml. The correlation coefficient (R2) was 0.9967 (95% confidence interval: 0.9690-1.0; Figure 3)

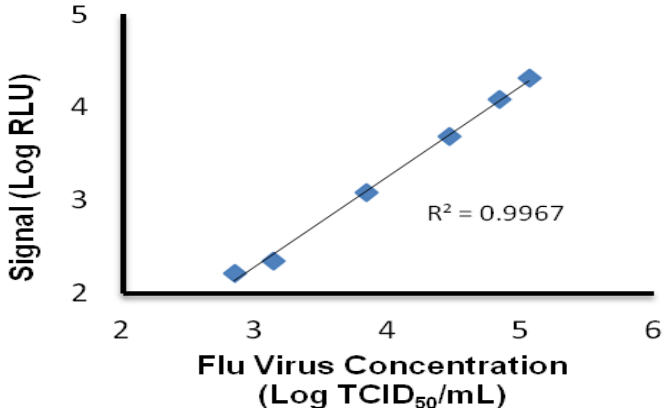

Fig. 3. Linearity and linear range of various influenza virus concentrations. Signal intensity (log RLU) was plotted against the flu virus concentration(log TCID50 /ml).

To evaluate the variability, two positive samples were tested every day, and each sample was repeated twice for 12 consecutive days. The results are shown as Table2. The test of all samples (95% confidence interval: 92.75% - 99.95%) can correctly diagnose whether there is influenza virus in the samples.

Table 2 . Repeatability of different samples

	Positive Sample 1			Positive Sample 2		Negative Sample
	1	2	3	4	5	6
Mean RLU(n = 48)	373	1009	3933	724	3357	101
SD	82	198	1003	228	352	34

%CV	21.91	19.68	25.50	31.49	10.48	33.59
% Positive	100	100	100	100	100	0

To evaluate the limit of detection (LOD), we tested 5 samples of influenza virus strains A/CA/07/2009 and A/NC/37/2009 for 20 times. The concentrations of the samples were close to the detection limit. The results are shown in Table 3. The diagnostic accuracy of positive influenza samples should be at least 95%, so the detection limits of A/CA/07/2009 and A/NC/37/2009 were 995 TCID50/mL and 953 TCID50/mL, respectively.

Table 3.LOD at various concentrations of influenza virus strains

	A/CA/07/2009			A/NC/39/2009	
	1	2	3	1	2
Concentrations (TCID50/mL)	663	995	1,326	953	1,271
Mean(S/CO)	0.98	1.58	1.96	1.15	1.87
% CV(S/CO)	13.81	7.98	6.59	8.36	3.45
% Positive	45%	100%	100%	100%	100%

To evaluate the performance of the assay in clinical settings, a clinical study was designed and conducted. The study was approved by the medical ethics committee at Jiangmen Wuyi Hospital of Chinese traditional medicine, and conducted according to the requirements of the China Food and Drug Administration (CFDA). From 25/01/2019 to 22/07/2019, 361 participants were enrolled in the study. The results are presented in Table4. The positive predictive value (PPV) was 100%, and the negative predictive value (NPV) was 98.90%. The colloidal gold reagent of Guangzhou Wondfo Biotech Co., Ltd., was used as reference reagent.

Table 4. The test results from clinical study.

		Results confirmed by colloidal gold		total
		Positive	Negative	
Results confirmed by this assay	Positive	90	3	93
	Negative	0	268	268
Total		90	271	361

Use nasopharyngeal swab to collect sample. Swab should go down to nasopharynx to collect adequate sample. Tenderly rotate the swab 5 rounds. To ensure adequate sample collection, repeat the same operation in the other cavity. The samples were tested at the end of each day. The test results were recorded and saved according to relevant guidelines and regulations.

4 CONCLUSION

High mutation rate and large number of variants make the diagnosis of influenza a difficult problem. Neuraminidase is a conserved enzyme of influenza virus, which is not susceptible to virus mutation; therefore, it is an ideal influenza diagnostic marker. The instrument described in this paper is based on a luciferase-based biochemiluminescence assay, and can diagnose influenza by detecting neuraminidase. The instrument has no liquid circuit design, so it has the advantages of simple structure, high stability and low cost [14]. Experimental results show that the instrument and reagent can be suitable for use in POCT settings.

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