ELECTROCHEMICAL DETECTION OF INFLUENZA VIRUS USING CDS QUANTUM DOTS AND 3D PRINTED CHIP

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1. INTRODUCTION

Influenza is one of the most frequently respiratory and febrile disease, which cause about 500 000 deaths worldwide every year. Influenza infections are mostly located into the seasonal epidemics or less frequently pandemics [1], the biggest one was Spanish flu in 1918. There is a wide spectrum of potential pandemic agents, but Influenza is the most probable, due to constant mutational changes of influenza surface protein (antigens): neuraminidase (NA) and hemaglutinin (HA). Both of them play the key role in the infection [2]. HA is responsible for virus entry in the host cell. NA allows releasing of new formed virion from host cell after replication. HA is able to induce immune response and represents the major component of influenza vaccines [3]. Rapid and sensitive method for influenza virus detection is still required.

2. MATERIALS AND METHODS

2.1. Labelling of influenza vaccine HA by CdS QDs

CdSQDs were prepared with a slightly modified method published by Duan J. et al. [8]. HA from vaccine was labelled by CdS. HA-CdS was used for isolation after further treatment. Sheme of isolation proces was described on Fig.1.

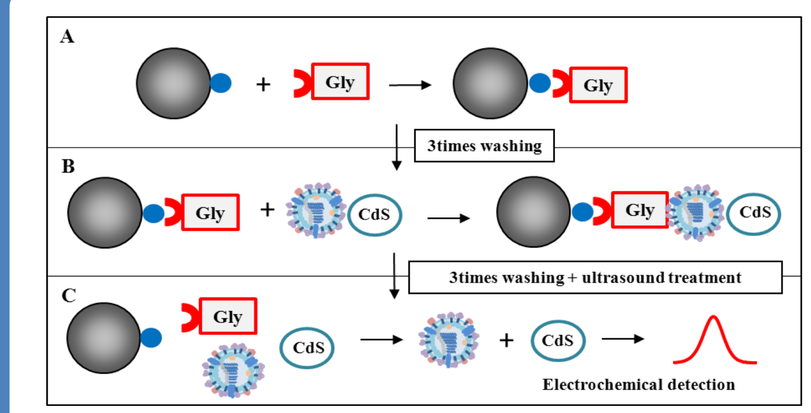

Fig. 1: Scheme of a fully automated method of detection influenza virus hemaglutinine (HA. (**A**) Biotinylated glycan binds to streptavidin modified MPs, (**B**) HA-CdS complex binding on glycan conjugated MPs (**C**) releasing of HA and cadmium(II) using ultrasound and their electrochemical detection.

2.2. Methods for electrochemical detection of HA, CdS and HA-CdS complex

HA, CdS and HA-CdS complex was detected by electrochemical analysis, which was performed with a 663 VA Stand instrument (Metrohm, Switzerland), in cooled cell, set at 4 °C, by three electrode set up. Software GPES 4.9 was used for data analysis. Prior to measurements samples were deoxygenated by argon (99.99%, for 120 s).

Cd (CdS) itself and from HA-CdS complex was detected by differential pulse voltammetry Anodic stripping technique (AST DPV) (Fig.2/B). Acetate buffer was used as electrolyte. The parameters were: purging time 100 s; deposition potential -0.85 V; equilibration time 2 s; modulation time 0.057 s; interval time 0.2 s; initial potential -0.85 V; end potential -0.4 V; step potential 0.005 V; modulation amplitude 0.0250 V.

HA itself and HA from HA-CdS complex was detected by Brdicka reaction [9] (Fig.2/C). Adsorptive transfer technique connected with differential pulse voltammetric measurements (AdT DPV). The parameters were as follows: initial potential of -0.7 V; end potential of -1.75 V; modulation time 0.057 s; time interval 0.2 s; step potential 0.002 V; modulation amplitude 0.025V.

2.3. Isolation of HA-CdS by glycan modified MPs using Robotic pipetting station

Automated pipetting station, ep-Motion 5075 (Eppendorf) was used for HA-CdS isolation, prior to electrochemical analysis. Isolation procedure was computer controlled, program sequence was edited and monitored by pEditor 4.0. Scheme of isolation procedure is showed on Fig. 1. Effect of different conditionns on isolation process was investigated (Fig.2). Parametres of electrochemical detection was described in 2.2.

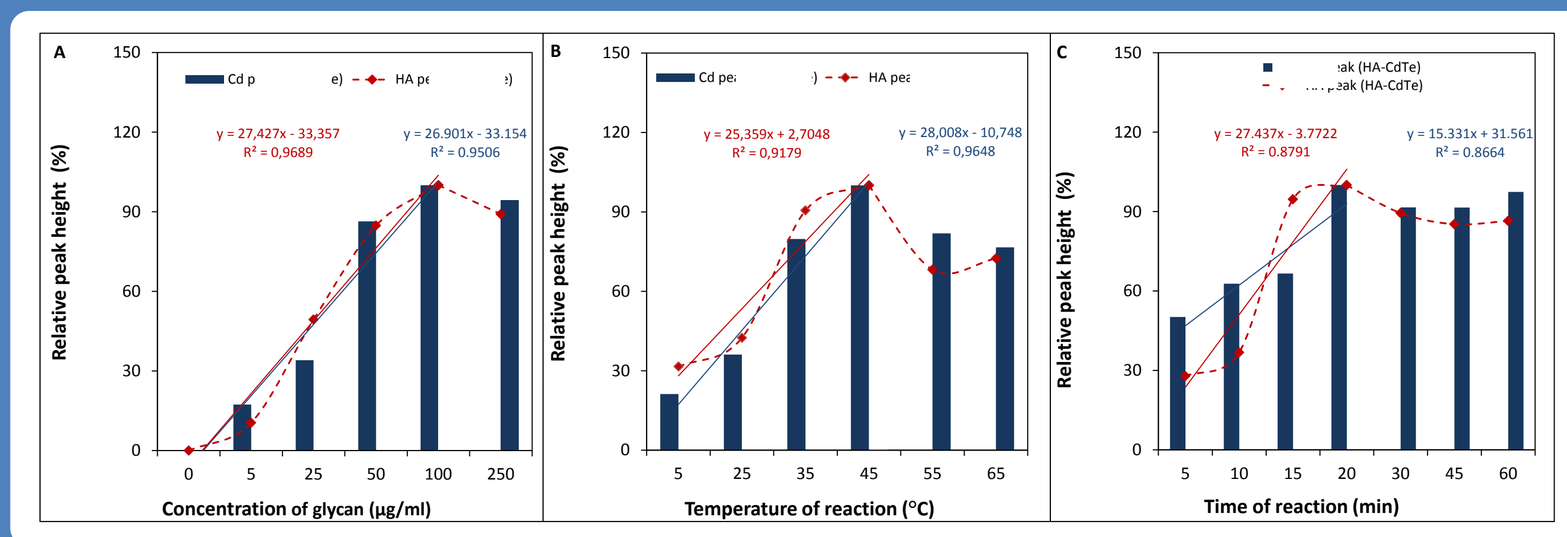

Fig.2: Optimization of isolation procedure. (**A**) Dependence of relative Cd/HA peak height (%) on concentration of glycan (µg/ml). (**B**) Dependence of relative Cd/HA peak height on temperature of reaction between HA-CdS and glycan modified MPs. (**C**) Dependence of relative Cd/HA peak height (%) on time of reaction(s) (HA-CdS + glycan). Dependence of relative Cd peak height (%) on optimized condition (blue column). Dependence of relative HA peak height (%) on optimized condition (red rhomb). For electrochemical methods and parameters see **2.2.**

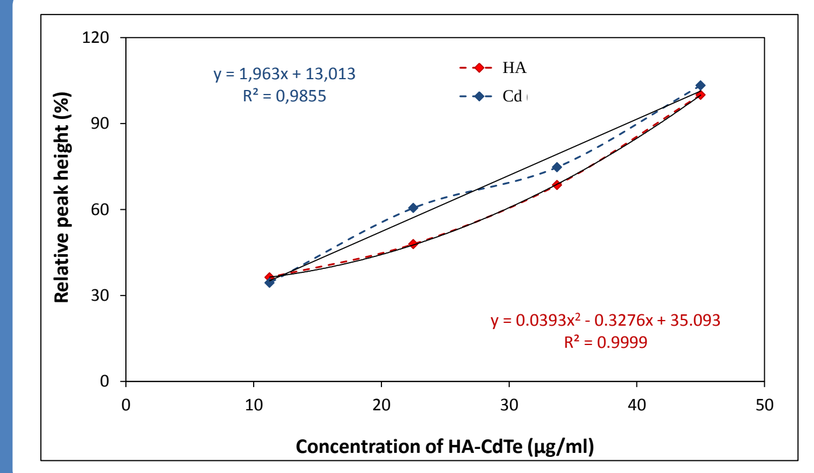

Fig.3: Detection of isolated HA-CdTe by electrochemical analysis . Dependence of relative peak height (%) (Cd peak – blue colour, HA peak – red colour) on concentration of isolated HA-CdTe complex (µg/ml

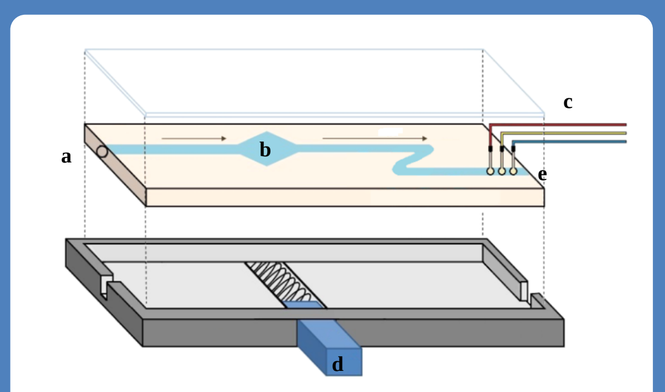

Fig.4: Scheme of 3D printed chip. a influx, b reaction chamber, c three-electrode set-up, d magnetic pad, e eflux.

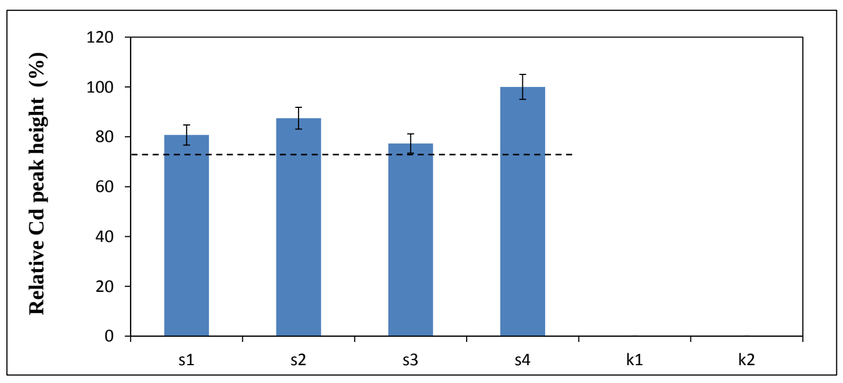

Fig.5: Effect of isolation and detection procedure on the real sample detection. Real sample is inactivated avian influenza virus H5N1(295/Turkey/Canda/6213/66) labelled with CdS. The results were obtained through the electrochemical detection of Cd, using 3D chip. S1 – S4 are the same samples and K1 and K1 are the negative controls.

3. RESULTS AND DISCUSION

In this study we described two steps assay for isolation and detection of influenza vaccine antigens (HAs). The first step was the isolation of HA-CdS using glycan modified MPs, the second step was electrochemical analysis, accompanied by spectroscopic detection. Two techniques of DPV were used for detection of isolation product. Conditions of isolation product was optimized using automated pipetion station (ep Motion 5075). Effect of different conditionns was placed as partial aim of study. And the optimal conditions were selected, based on electrochemical detection of Cd and HA peak of isolation product: concentration of glycan 100 µg/ml (**Fig.2/A**), temperature of isolation reaction 45°C (**Fig.2/B**) and time of reaction 20 minutes (**Fig.2/C**). Under optimized conditions were tested vaccine HA labelled by CdS at various concentrations of HA-CdS complex. Results of isolation was detected electrochemically as dependence of Cd and HA peak on concentration of isolated HA-CdTe complex (**Fig.3**). Than optimized conditions from isolation using ep Motion 5075 was placed in 3D chip and real influenza samples were tested (inactivated influenza virus H5N1). Shcema of 3D chip was described on Fig.4. Results from 3D chip assay are presented on Fig. 5. It is obvious the good accordance between samples and negative control, which gives no signal.

4. CONCLUSION

We designed and optimized method for automated isolation of influenza hemaglutinine labeled by CdTe. For detection electrochemical and spectroscopic analysis were used. It was observed the effect of glycan concentration, time and temperature of glycan and HA-CdTe mixture detected by HA and Cd peak height. We demonstrate, that optimized isolation procedure coupled with electrochemical and spectroskopic analysis, is usefull tool for real samples detection. Our platform may be applied for analysis of other pathogens, based on specific receptor binding and magnetic isolation.

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

ACKNOWLEDGEMENTS

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