

# Hollow nanogold microsphere-signalized lateral flow immunodipstick for the sensitive determination of the neurotoxin brevetoxin B

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**Abstract** We report on a new membrane-based lateral-flow assay for the rapid determination of the algal biotoxin brevetoxin B (BrevTox) that can be found in fish. Hollow nanogold microspheres (HGMS) with an average size of 45 nm were synthesized by using the reverse micelle method, followed by functionalization with monoclonal mouse anti-BrevTox antibody (mAb-HGMS). They served as the signaling reagent. Bovine serum albumin-BrevTox conjugate and rabbit anti-mouse IgG antibody were manually spotted on the nitrocellulose membrane and served as reagents for the test line and control line, respectively. Both spherical gold nanoparticle-labeled anti-BrevTox antibody (mAb-AuNP) and mAb-HGMS conjugate were investigated with respect to their suitability as signal-transduction tags, and the latter was found to give improved analytical features. The HGMS-based labeling method was then also studied in terms of performance in the immunodipstick assay. Under optimal conditions, the visual detection limit (cut-off value) of the mAb-AuNP based assay is  $1.5 \text{ ng mL}^{-1}$ , while the sensitivity of the mAb-HGMS based assay is as low as  $0.1 \text{ ng mL}^{-1}$ . Yes/no decisions can be made within 10 min without the need for expensive instrumentation. The results for the analysis of target BrevTox in spiked fish samples showed a good correlation with data obtained with the commercial ELISA. Importantly, the assay gave no false negative results.

**Keywords** Hollow nanogold microsphere · Brevetoxin B · Lateral-flow immunodipstick · Biotoxin

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## Introduction

Marine biotoxins are poisons that are produced by certain kinds of microscopic algae that are naturally present in marine waters, normally in amounts too small to be harmful [1]. Molluscan shellfish are filter feeders and ingest any particles, both good and bad, that is in the surrounding water [2]. Unlike most other biohazards, biotoxins do not replicate and, in some senses, are more analogous to chemical toxins. Proper handling and disposal of biotoxins pose special challenges but yet are vital steps toward the protection of laboratory and service personnel [3]. Immunological method, based on a specific antigen-antibody recognition event, has become the predominant analytical techniques for quantitative detection of biotoxin [4, 5]. Various methods and strategies have been utilized for the monitoring of biotoxins, e.g. by using quartz crystal microbalance [6], surface plasmon resonance [7], fluorescence [8], electrochemistry [9], immunochromatography [10], and chemiluminescence assay [11]. Despite the high sensitivity of conventional immunoassay methods, they have some limitations such as expensive instrumentations, sample multi-step separations and washing, skilled requirement, and a relatively long analysis time. An alternative immunosensing strategy that is based on simple and inexpensive approaches that do not require tedious and time-consuming sample preparation and cleanup steps would be advantageous [12, 13].

Unbeatable as to simplicity and speed of the technique, membrane-based immunoassays such as lateral-flow device and flow-through format tests have recently gained increasing attention in different fields including environmental monitoring, food safety, and clinical diagnosis, since it is inexpensive, rapid, and portable, and do not require technical skill to perform [14]. The lateral flow immunoassay is used in commercial pregnancy detection and is an accepted point-of-care testing technique [15]. The qualitative or semi-quantitative

determination, for example, a one-step test can be performed within a few minutes without the need of instrumentation and additional chemicals [16]. Furthermore, results are interpretable by non-specialists. Typically, gold colloids were extensively used for development of lateral-flow immunodipstick. Li et al. utilized gold nanoparticle-labeled antibody as the signal tag for rapid and quantitative detection of clenbuterol in swine urine based on an immunochromatographic assay [17]. Unfavorably, the method often suffers from poor quantitative discrimination and low analytical sensitivity. To tackle this limitation, Zhang et al. synthesized lanthanide chelate-loaded silica nanoparticles to label the primary antibody for the development of fluorescent immunochromatographic strip [18]. To the best of our knowledge, however, most of the reported immunodipstick assays nowadays were employed gold colloids as signal-transduction tags. Improved analytical properties could be obtained using more sophisticated analytical devices, e.g. fluorescence, for the development of lateral-flow immunodipstick assays [19, 20]. Unfavorably, the fluorescence immunodipstick assays largely weakened the inherent advantages of the lateral-flow immunodipstick assay, e.g. no instrumentations.

For successful development of user-friendly tests accompanying sufficient sensitivity without the need the external instrumentation, the signal-transduction labels (tags) are very important because the lateral-flow immunodipstick is basically designed for visual inspection [21, 22]. The detector reagents range from the most commonly used colloidal gold to dye-containing liposomes [23]. Gold colloids have been extensively employed for the labeling of different biological receptors, such as proteins, IgG and enzyme due to their inherent advantages, such as easy preparation and good biocompatibility [24]. Just as the visible color derives from the integration of the individual nanoparticle in the test/control lines, the visible color was often difficultly distinguished to some extent. The major reasons are the low signal intensity and poor quantitative discrimination of the color-formation reaction based on label accumulation. To further highlight the advantages of gold colloids, our motivation in this work is to synthesize hollow nanogold microspheres for the label of primary antibody in order to improve the sensitivity of the lateral-flow immunodipstick assays.

Brevetoxin B (BrevTox), as a neurotoxin produced by algae, can cause intoxication and even mortality through consumption of brevetoxin-contaminated shellfish, and affect respiratory irritation through aerosol exposure at coastal areas. Using BrevTox as a model biotoxin, herein we design a new membrane-based lateral-flow immunodipstick assay for the screening of BrevTox using hollow nanogold microsphere (HGMS) as the signal-transduction tag. The assay is based on the reaction of HGMS-labeled capture antibody (mAb-HGMS) with BSA-BrevTox conjugate and rabbit anti-mouse IgG on the test/control lines, respectively. In the absence of

target BrevTox, the HGMS-labeled anti-BrevTox antibody flows through the test line accompany with the detection solution, and reacts with BSA-BrevTox conjugate immobilized on the control line, thus resulting in the visible pink color at the test. Meanwhile, the excess mAb-HGMS goes ahead and reacts with IgG, thereby forming two visible pink color bands at the test line and control line. Upon target BrevTox introduction, the formed immunocomplex with the HGMS crosses through the test line, and reacts with IgG immobilized on the control line, thus resulting in the visible pink color at the control line. The intensity of the appeared color at the test line is directly proportional to the concentration of target BrevTox in the sample. The aim of this study is to exploit a new signal-enhanced lateral flow immunodipstick for low-concentration target analyte with high sensitivity by using the bionanotechnology.

## Experimental

### Reagents and chemicals

Individual standard stock samples of brevetoxin B (BrevTox, purity  $\geq 98\%$  by HPLC) were purchased from Express Technol. Co., Ltd (Beijing, China, [www.express-cn.com](http://www.express-cn.com)). Monoclonal mouse anti-BrevTox antibody and BrevTox-bovine serum albumin (BrevTox-BSA) conjugate were synthesized by Dingguo Biotechnol. Co., Ltd (Beijing, China, [www.dingguo.com](http://www.dingguo.com)). Anti-mouse IgG (whole antiserum, produced in goat,  $1.0\text{ mg mL}^{-1}$ ) was purchased from Sigma-Aldrich (China, [www.sigmaaldrich.com](http://www.sigmaaldrich.com)). Tween 20, gold (III) chloride trihydrate ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ , 99.9 % metals basis), sodium citrate tribasic dihydrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ), bovine serum albumin (BSA, 96–99 %), and bis-(2-ethylhexyl) sodium sulfosuccinate (AOT) were got from Sinopharm Chem. Re. Co., Ltd (Shanghai, China, [www.sinoreagent.com](http://www.sinoreagent.com)). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system ( $\geq 18\text{ M}\Omega$ , Milli-Q, Millipore, [www.millipore.com](http://www.millipore.com)) was used in all runs. 0.1 M phosphate-buffered saline (PBS, pH 7.4) was prepared by adding 12.2 g  $\text{K}_2\text{HPO}_4$ , 1.36 g  $\text{KH}_2\text{PO}_4$ , and 8.5 g NaCl in 1,000 mL deionized water.

Cellulose absorbent paper, glass fiber and nitrocellulose membrane (pore size,  $0.2\ \mu\text{m}$ ) were provided from Whatman GmbH (Dassel, Germany, [www.whatman.com](http://www.whatman.com)). PVC sheets, adhesive tape and filter paper were purchased from Shanghai KinbioTech. Co., Ltd (China, [www.goldbio.com](http://www.goldbio.com)).

### Preparation of hollow nanogold microsphere (HGMS)

The HGMS was synthesized by using reverse micelle method according to our previous report with a little modification

[25]. Initially, two solutions were prepared as follows: solution A including 200  $\mu\text{L}$  of 50 mM  $\text{CaCl}_2$  and 15 mL of 0.1 M AOT isooctane suspension, and solution B containing 200  $\mu\text{L}$  of 50 mM  $\text{Na}_2\text{CO}_3$  and 15 mL of 0.1 M AOT isooctane suspension. Afterward, solution B was dropped into solution A with violent stirring to form colloidal  $\text{CaCO}_3$ . Following that, 100  $\mu\text{L}$  of 5 wt %  $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$  ( $\sim 12$  nmol) was added into the reverse micellar solution drop by drop. After adequately stirring, 100  $\mu\text{L}$  of 50 mM hydrazine hydrate aqueous solution was added into the resulting suspension. During this process, the Au(III) coated on the surface of  $\text{CaCO}_3$  colloids was reduced to zero-valent  $\text{Au}^0$ . With the progressive addition of hydrazine hydrate to reverse micelles, the solution acquired a fawn to red color due to the formation of gold colloids. After stirring for 1 h at room temperature (RT), 3 mL of absolute ethanol was added and stirred for 10 min, which resulted in the complete breakdown of reverse micelles with the formation of two immiscible layers of aqueous ethanol and iso-octane. The ethanol was carefully removed by using a separating funnel. The particles thus obtained were washed four times with iso-octane and centrifuged to remove any residual AOT. The pelleted particles were then dispersed in 10 mL water by vigorous stirring for 30 min and the dispersed system was dialysed against distilled water for 2 h using a 12 kDa cut-off dialysis bag. Finally, the HGMS was formed upon addition of 0.1 M HCl into the resulting suspension.

#### Preparation of mAb-HGMS conjugate

Initially, 120  $\mu\text{L}$  of 0.5 mg  $\text{mL}^{-1}$  mAb antibody (optimized) was added into 10 mL of HGMS suspension ( $C_{[\text{Au}]} \approx 0.24$   $\mu\text{M}$ ) (pH 9.3) with gently stirring, and then incubated for 4 h at RT. The resulting mixture was centrifuged at 10,000 g for 15 min at 4°C in order to remove the excess proteins. Following that, the obtained pellet was re-suspended in 1.0 mL of 2 mM sodium carbonate solution containing 8 wt % (w/v) sucrose, 1 wt % BSA and 0.1 % sodium azide, pH 9.2, and stored at 4°C for further use ( $C_{[\text{Au}]} \approx 2.4$   $\mu\text{M}$ ).

#### Preparation of the lateral-flow immunodipstick strip

The preparation and assembly of lateral-flow immunodipstick strip is described as follows and schematically illustrated in Scheme 1. Sample pad made of glass fiber was saturated with 0.1 M PBS (pH 7.4) containing 0.1 % Tween-20 and 1 wt % BSA, which was dried at 60°C and kept in a desiccator at RT until use. The mAb-HGMS conjugate pad was prepared by immobilizing mAb-HGMS conjugate on a glass fiber. Initially, the glass fiber was treated with 0.1 % Tween-20 for 24 h and dried for 30 min at 60°C. The resulting glass fiber was dipped in the mAb-HGMS suspension, then lyophilized and

stored in a desiccator at 4°C until use. The nitrocellulose membrane ( $4 \times 1.5$  cm) on which capture reagents, e.g. BSA-BrevTox and IgG, were immobilized on the test strip and control strip, respectively. At a distance of 0.5 cm from top of the membrane, a band of secondary antibodies (undiluted goat anti-mouse IgG) was manually spotted as the control line at a volume of 1.0  $\mu\text{L cm}^{-1}$  membrane width, and analogously, BSA-BrevTox (1.0 mg  $\text{mL}^{-1}$ ) was applied as test line at a distance of 0.5 cm from bottom. After the membrane was dried for 1 h at RT, it was blocked with 2.5 wt % BSA in pH 7.4 PBS for 30 min on a shaker and again dried for 1 h at RT, and stored in a desiccator at 4°C. 100 % pure cellulose fiber with a high absorbent volume capacity was used as the absorbent pad. The as-prepared membranes were assembled onto a plastic backing card. The nitrocellulose membrane was pasted onto the middle of the plastic card. The conjugate pad was pasted on the card by overlapping 1.8 mm with the nitrocellulose membrane. Absorbent pad and sample pad were pasted on the two sides of the strip. The master card was cut to 4 mm width. The formed lateral flow immunodipstick strip was stored in a desiccant at RT when not in use.

#### Preferred position of Scheme 1

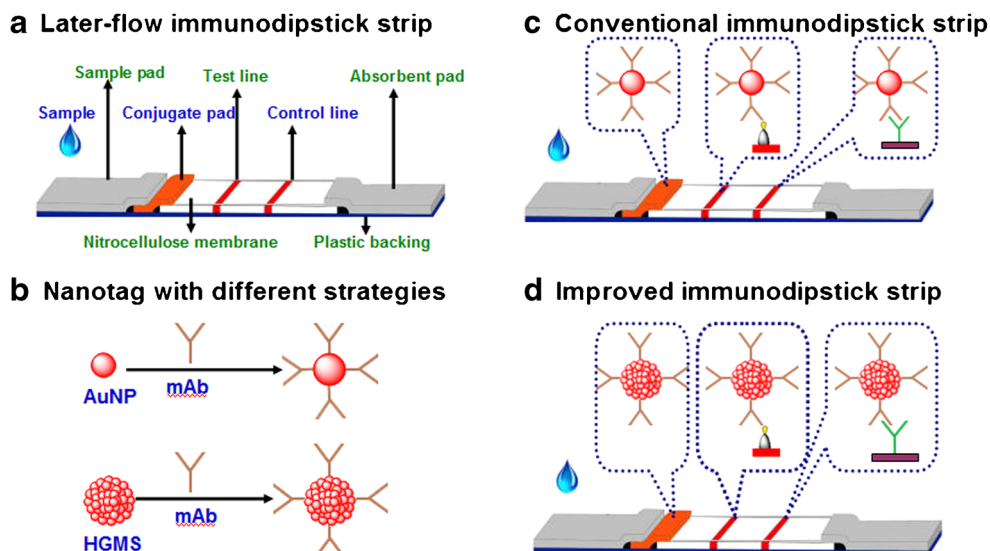
#### Immunodipstick procedure

100  $\mu\text{L}$  of BrevTox standards/samples with various concentrations was pipetted onto the sample pad of the immunodipstick test strip. Upon addition of the liquid standard/sample solution, the mAb-HGMS coated on the conjugate pad was dissolved and flowed with the sample solution toward the membrane on which capture reagents were immobilized. Different intensities of red color at test line and control line could be observed by naked eye. The color was recorded  $\sim 10$  min after addition of target analyte on the immunodipstick strip.

#### Samples and preparation procedure

Mollusk standards were prepared similar to our previous report [4] as follows: Seafood samples including *Musculista senhousia*, *Sinonovacula constricta*, and *Tegillarca granosa* were purchased from the local Carrefour supermarket (Fuzhou, China). 5 g of minced sample tissue was initially placed in a centrifuge tube, then 20 mL of dimethyl sulfoxide (50 %, w/v) was added, and then the mixture was centrifuged for 10 min at 2,500 g. The resulting supernatant was passed sequentially through a 100-nm nylon mesh, no. 1 filter and a GF/B filter (Whatman) in turn (*Note*: The extract color was slightly opalescent yellow). Following that, BrevTox standards with various levels were spiked into the supernatant, respectively.

**Scheme 1** Schematic illustration of (a) the lateral-flow immunodipstick assay and (b) different signal-transduction tags. Principle of BrevTox detection using (c) conventional and (d) improved immunodipstick strip



## Results and discussion

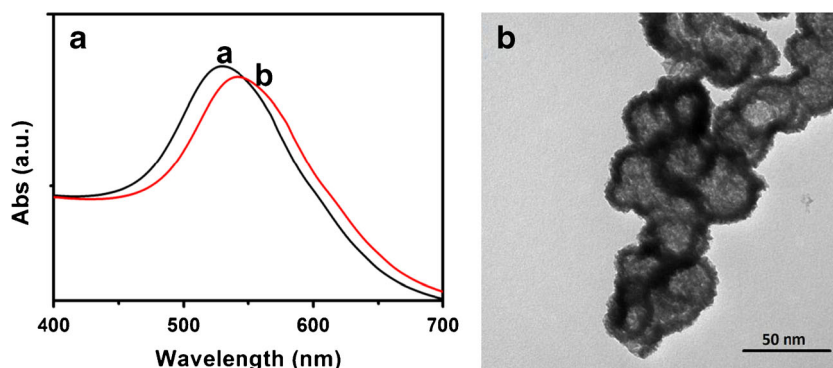
Principle and construction of HGMS-based lateral-flow immunodipstick assay

Scheme 1 gives the fabrication process of the HGMS-based lateral-flow immunodipstick test strip. Hollow nanogold microspheres were synthesized by using the reverse micelle method. The as-synthesis HGMS was characterized, as described in detail in our previous paper [25]. The association of mAb antibody onto the HGMS was possibly due to the interaction between cysteine or  $\text{NH}_3^+$ -lysine residues of protein and gold nanoparticles [26]. The successful synthesis of mAb-HGMS was qualitatively characterized using UV-vis absorption spectroscopy. As seen from Fig. 1a, the spectrum of pure HGMS (curve 'a') exhibited a characteristic plasmon absorption peak at 529 nm. After the interaction between HGMS and mAb, the plasmon absorption peak shifted from 529 nm to 537 nm (curve 'b'), indicating the formation of mAb-HGMS [26]. Fig. 1b shows high-resolution transmission electron microscope (HRTEM) of the as-synthesized HGMS, which displays a well dispersion in the distilled water with an

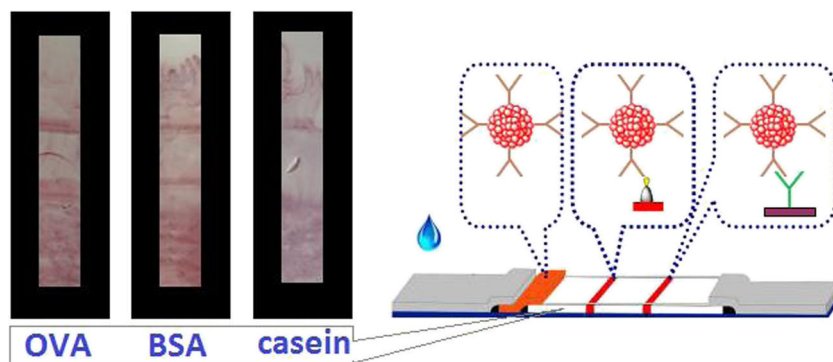
average size of 45 nm. Significantly, we could also observe that the HGMS contained a large number of single gold nanoparticles. The aggregation of gold nanoparticles was expected to enhance the sensitivity of the lateral-flow immunodipstick assay.

The principle of the immunodipstick assay is based on a sandwich-type immunoassay format. When BrevTox standard/sample solution was added onto the sample pad, the liquid solution was moved forward to the absorbent pad. The mAb-HGMS on the conjugate pad was dissolved and move together with liquid solution. In the absence of target BrevTox in the sample, the unreacted mAb-HGMS reacted with BSA-BrevTox immobilized on the test line. Excess mAb-HGMS would be moved continuously to the control line, which was captured by the immobilized goat anti-mouse IgG. Two red bands on the test line and control line would appear due to the accumulation of red colored gold colloids. In the presence of target BrevTox, almost all the binding sites on the mAb-HGMS were occupied, which could not reach the control line, thus resulting in only one strong red band at the test line. The positive or negative results as obtained by the dipsticks relied on visible pink color of the

**Fig. 1** a UV-vis absorption spectra of (a) the as-prepared HGMS and mAb-HGMS; b HRTEM image of the as-prepared HGMS



**Fig. 2** Photo images of the HGMS-based immunodipstick strips by using different blocking materials toward  $0.05 \text{ ng mL}^{-1}$  BrevTox



test and control lines. That is to say, if the level of BrevTox in the sample was lower than the cutoff value (sample considered negative), both lines were colored. In contrast, only one colored test line appeared if the concentration of BrevTox in the sample was higher than the cutoff value (sample considered positive). Concluding, the control line should be always visible regardless of the concentration of the analyte.

#### Optimization of experimental conditions

In this work, glass fiber and nitrocellulose membrane were used for the construction of the immunodipstick strip. Typically, they are naturally hydrophobic materials, which might cause the non-specific absorption toward the mAb-HGMS and other proteins. To investigate the point, three blocking reagents including BSA, ovalbumin (OVA) and casein were studied for their ability to block non-specific binding on the immunodipstick strip by using  $0.05 \text{ ng mL}^{-1}$  as an example. As seen from Fig. 2, two unambiguous red bands were observed at the test line and control line when using 2.5 wt % BSA as the blocking agent (strip 'a'). In contrast, a large-scale red region could be appeared except for the test/control lines when using OVA (strip 'b') and casein (strip 'c') as the blocking agents, respectively. The reason might

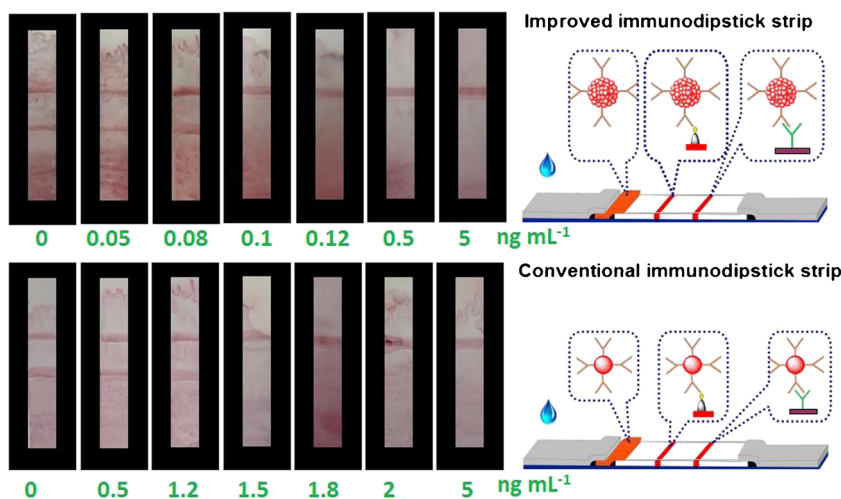
be most likely a consequence of the non-specific absorption toward mAb-HGMS and BrevTox/mAb-HGMS. Thus, 2.5 wt % BSA was used as the blocking agent of the lateral-flow immunodipstick strip in the work.

In addition to the blocking agent, the analytical properties of the membrane-based immunodipstick assay can be also affected by other parameters, e.g. the type and pore size of the membrane and type of absorbent pad. However, the main aim of this work was to investigate the effect of the as-synthesized HGMS on the sensitivity of the immunodipstick assay in comparison with that of conventional gold nanoparticles. Thus, some classical materials most commonly used for the immunodipstick assays, e.g. nitrocellulose membrane ( $0.2 \mu\text{m}$  pore size) and cellulose fiber pad, were also applied in this study. As is well known, nitrocellulose membranes generally do not cause high background and flow obstruction, while the cellulose fiber is a good absorbent, and has a high volume capacity.

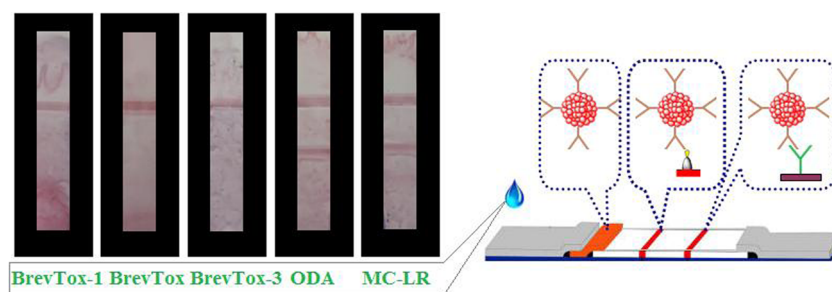
#### Cutoff value of the immunodipstick assay

To rapidly evaluate the positive/negative samples, a semi-quantitative result, which can exactly provide a yes/no

**Fig. 3** Photo images of lateral-flow immunodipstick assay toward BrevTox standards with various concentrations using different signal-transduction tags: (top) the as-synthesized mAb-HGMS and (bottom) spherical gold nanoparticle-labeled mAb



**Fig. 4** The specificity of HGMS-based lateral-flow immunodipstick assay against 5 ng mL<sup>-1</sup> BrevTox-1, 5 ng mL<sup>-1</sup> BrevTox, 5 ng mL<sup>-1</sup> BrevTox-3, 5 ng mL<sup>-1</sup> ODA and 5 ng mL<sup>-1</sup> MC-LR



response indicating that target BrevTox is present or not above the visible detection limit (cut-off value), is acceptable. In the present paper, the cutoff value was defined as the lowest BrevTox concentration, which inhibited apparent color development at the control line. Under optimal conditions, BrevTox standards with various concentrations were detected by using the developed immunodipstick strip. As indicated from Fig. 3(top), the typical red lines at the test regions were visualized, and the intensity of red color on the test lines enhanced gradually with the increase of target BrevTox concentration in the sample. When the concentrations of target BrevTox were  $\geq 0.1$  ng mL<sup>-1</sup>, the red bands at the test lines were almost disappeared. Thus, 0.1 ng mL<sup>-1</sup> BrevTox was defined as the cut-off value of the developed immunodipstick assay. For comparison, we also used pure gold nanoparticles as the detection element by using the same-concentration mAb (120  $\mu$ L, 0.5 mg mL<sup>-1</sup>) and the same-level gold (10 mL, 0.24  $\mu$ M). Experimental results indicated that the cut-off value of using pure gold nanoparticles as detection reagent was 1.5 ng mL<sup>-1</sup> (Fig. 3(bottom)). Although the system has not yet been optimized for maximum efficiency, the cut-off value of using the mAb-HGMS was 15-fold lower than that of pure gold nanoparticles. As speculated at the beginning, the insertion of multiple nanogold particles per binding event of mAb-HGMS to the pAb at the test line can

lead to higher sensitive immunological dipstick assays, obviously. The reason might be the fact that each HGMS contained many gold nanoparticles. When one antibody on the HGMS was reacted with target BrevTox, the whole HGMS could be carried over and exhibit a strongly visible band. More importantly, the LOD of the immunodipstick assay was almost comparable with commercialized BrevTox ELISA kit (0.05 ng mL<sup>-1</sup>, Abraxis LLC, USA), competitive-type ELISA (LOD: 0.6 ng well<sup>-1</sup>) [27], colloidal gold-based immunochromatographic assay (10 ng mL<sup>-1</sup>) [28], electrochemical immunoassay (10 pg mL<sup>-1</sup>) [29], and displacement-based electrochemiluminescence immunoassay (0.05 ng mL<sup>-1</sup>) [30] for BrevTox, respectively.

#### Specificity and reproducibility of the immunodipstick assay

The specificity of the HGMS-based immunodipstick assay was also investigated by spiking BrevTox-1, BrevTox, BrevTox-3, okadaic acid (ODA), and microcystin-LR (MC-LR) into the blank *Sinonovacula constricta* supernatant (5.0 ng mL<sup>-1</sup> used in this case). As seen from Fig. 4, the immunodipstick assay exhibited a high cross-reactivity (CR) of >95 % for BrevTox-1 and BrevTox-3, while no false compliant results were obtained for ODA and MCLR. The false positive results for BrevTox-1 and BrevTox-3 might be

**Table 1** Comparison of experimental results for BrevTox levels in spiked different seafood samples obtained by the HGMS-based immunodipstick assay and referenced ELISA

Sample type	Spiked level (ng mL <sup>-1</sup> )	Concentration (ng mL <sup>-1</sup> ); yes(+)/no(-)	
		ELISA (mean $\pm$ SD, n=3)	Immunodipstick (+/-) <sup>a</sup>
<i>Sinonovacula constricta</i>	0	No detected	---
	0.05	0.051 $\pm$ 0.012	---+
	0.5	0.49 $\pm$ 0.08	+++
	2.0	2.1 $\pm$ 0.12	+++
	0	No detected	---
<i>Musculista senhousia</i>	0.05	0.054 $\pm$ 0.022	++
	0.5	0.51 $\pm$ 0.09	+++
	2.0	1.98 $\pm$ 0.23	+++
	0	No detected	---
	0.05	0.053 $\pm$ 0.019	++
<i>Tegillarca granosa</i>	0.5	0.48 $\pm$ 0.11	+++
	2.0	2.12 $\pm$ 0.23	+++

<sup>a</sup> '+': positive (i.e. BrevTox level was higher than 0.1 ng mL<sup>-1</sup>); '-': negative (i.e. BrevTox level was lower than 0.1 ng mL<sup>-1</sup>)

ascribed to the fact that the used anti-BrevTox antibody has a high cross-reaction (CR) with BrevTox-1 and BrevTox-3. Hence, the specificity and anti interference of the immunodipstick assay were acceptable.

The batch-to-batch reproducibility was also studied by using different-batch HGMS for the determination of various-concentration BrevTox. The evaluation was carried out by calculating the cutoff value for each batch. Experimental results revealed that all cut-off values in these cases ( $n=9$ ) were  $0.1 \text{ ng mL}^{-1}$  BrevTox. Hence, the reproducibility of the as-prepared HGMS was satisfactory.

#### Screening of real samples and intralaboratory validation

To further investigate the possibility of the newly developed technique to be applied for testing of real samples, 12 spiked seafood samples with different-concentration BrevTox, such as *Sinonovacula constricta*, *Musculista senhousia* and *Tegillarca granosa*, were assessed by using the HGMS-based immunodipstick assay and commercialized BrevTox ELISA kit (Abraxis LLC, USA) as a referenced method. The spiked procedure was described in detail in the Experimental section. The results are summarized in Table 1. As seen from Table 1, all the samples which concentrations were higher than  $0.1 \text{ ng mL}^{-1}$  BrevTox as determined by ELISA were tested positive (+) by the immunodipstick assay, while 13 samples were negative (-), i.e. concentration was less than  $0.1 \text{ ng mL}^{-1}$  BrevTox. More significantly, the sensitivity and specificity rates of the immunodipstick assay were also evaluated by using these data (Eqs. i and ii). The sensitivity and specificity of a qualitative method are defined as the ability to detect truly positive ( $tp$ ) samples as positive and truly negative ( $tn$ ) samples as negative, respectively  $yy$ . The parameters can be calculated as:

$$\text{Sensitivity rate} = \frac{\text{test positives}}{\text{total number of known positives}} = \frac{tp}{tp + fn} \quad (1)$$

$$\text{Sensitivity rate} = \frac{\text{test positives}}{\text{total number of known negatives}} = \frac{tp}{tn + fp} \quad (2)$$

where  $fn$  and  $fp$  are false negative and false positive test samples, respectively. The immunodipstick assay had a sensitivity rate of 100 % ( $n=18$ ) and a specificity rate of 72.2 % ( $n=18$ ) (Table 1). No false compliant (i.e. false negative) results but 27.8 % of false noncompliant (i.e. false positive) results were obtained. The latter will require ELISA confirmation after the

screening. Thus, the developed immunodipstick assay could be considered as an optional scheme for rapid monitoring of target BrevTox in the biological samples.

#### Conclusions

In summary, this work demonstrates a new approach toward the development of higher sensitive immunodipstick assay using the as-prepared HGMS as the signal-transduction tag. Experimental results indicated that the HGMS-based immunodipstick assay was as simple as conventional colloidal gold-based strip test. More importantly, the cut-off value of using the HGMS was about 20-fold lower than that of using gold colloids. Moreover, the results can be achieved within 10 min without expensive equipment. Thus, the HGMS-based immunodipstick assay can provide an alternative tool for sensitive, rapid, convenient and semi-quantitative detection of the analyte on-site. More impressively, the system opens a new opportunity for the detection of specific small molecules. Future work should be focused on other small molecules by controlling the target antibody, thus demonstrating the versatility of the assay scheme.

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