

# Application of antibody –labeled magnetosomes in quantitative detection of HBsAg with chemiluminescence–immunoassays

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**Abstract** Combined with chemiluminescence–immunoassays, HBsAg in human body can be quantitatively detected by using antibody–labeled magnetosomes. 50 serum samples from hepatitis B patients are detected by using double antibody sandwich method which includes using monoclonal antibody to label magnetosomes, polyclonal antibody to label alkaline phosphatase, dioxetanephosphate as luminous substrates. Results show that 20 people are hepatitis B patients, 30 people are hepatitis B carriers merely. Besides, compared with ELISA, 50 random human serum samples are detected. Among 4 kinds of label methods, the one using double function reagent, SPDP, to couple antibody and magnetosomes is the best. And then the method of quantitative detection of HBsAg with chemiluminescence–immunoassays is established. The threshold of sensitivity of this method in detecting antigens is 0.1 ng/ml, which completely accords with the results obtained by using standard specific serum samples that provided by National Institute For The Control of Pharmaceutical and Biological Products. Among 100 clinical samples detected by this method, there are 20 hepatitis B patients and 30 hepatitis B carriers, which is 100% consistent with the results obtained by ELISA. While used to detect 50 random human serum samples, this method is better than ELISA because of its higher sensitivity. Results show that this method can be used in the early detection of HBsAg because of its high sensitivity, strong–specificity, good quantitative accuracy.

**Keywords:** magnetosome, chemiluminescence, ELISA, HBsAg

Since discovered by Blackmore in 1975<sup>[1]</sup>, magnetotactic bacteria has been attached much importance to by many scientists because of their magnetotaxis caused by the intracellular magnetosomes, nanoparticles sensitive to magnetic field.

The magnetosomes in *Magnetospirillum gryphiswaldense* cells, of which the major composition is Fe<sub>3</sub>O<sub>4</sub>, are characterized by narrow size distributions, membrane coating, large specific surface area, and uniform crystal habits that they can be used as carriers for antibody, enzyme, drug and gene<sup>[2–5]</sup>. At the beginning of 90's last century, Matsunaga forecasted that magnetotactic bacterial magnetosomes would be one novel biological resource used in the application of high technique in the following decade. To these days, however, the applied investigation of magnetosomes are merely limited to the lab due to rigor requirement of magnetotactic bacteria for the nutrition and dis-

solved oxygen. In the previous research work, we solved the problems occurred in the mass culture by a submerged culture technique for *M. gryphiswaldense* MSR–1 in a 5 L oxygen –controlled auto –fermentor and followed purified magnetosomes. In this work, we quantitatively detected HBsAg and obtained ideal results by chemiluminescence–immunoassays, using purified magnetosomes coupled with monoclonal antibody of HBsAg.

## 1 Materials and Methods

### 1.1 Materials

Purified magnetosomes isolated from the cells of *M. gryphiswaldense* MSR–1 were prepared by Microbiology Department, College of Biological Sciences, China Agricultural University; Monoclonal antibody and polyclonal antibody of HBsAg, and kit of ELISA for HBsAg were supplied by Sino –American Biotechnology Co.; alkaline phosphatase (AP), SPDP, glutaraldehyde and sodium metaperiodate were purchased from Sigma Co.; Dioxetanephos-

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phate was purchased from Bio-Rad Co.; Luminoskan Ascent, Thermo Labsystems Co.; Standard serum samples were supplied by National Institute For The Control of Pharmaceutical and Biological Products; Clinic samples and random samples were provided by Clinical Laboratory, Central Hospital of Luoyang.

## 1.2 Methods

### 1.2.1 Preparation for monoclonal antibody of HbsAg

Ascites of monoclonal antibody of HBsAg was purified<sup>[7]</sup> and the purity of antibody was detected by SDS-PAGE.

### 1.2.2 Preparation for magnetosomes

Combined with weakly ultrasonic bathing, magnetosomes suspended and distributed in 0.01 M PBS (pH=7.4) were washed several times until they were purified.

### 1.2.3 The method of direct absorption

After dialyzed in 0.05 M carbonic acid buffer (pH=9.5), monoclonal antibody of HBsAg was added into magnetosomes pro rata that (the protein content of antibody)/(the dry weight of magnetosomes) was 1:1 and the mixture incubated at 37°C was intermittently stirred for 2 hours. Then the magnetosomes were absorbed to the bottom of the vessel by using a magnet and were washed three times by 0.01 M PBS after the supernatant was discarded. The magnetosomes were resuspended in 1% BSA dissolved in 0.01 M PBS and incubated at 37°C for 2 hours, intermittently stirring. After the suspension was centrifuged and the supernatant was discarded, the magnetosomes pellet was conserved at 4°C as a spare.

### 1.2.4 The method of labeling with glutaraldehyde

0.3 ml 1.25% glutaraldehyde solution prepared with 0.01 M PBS (pH=6.8) was added into 1 ml magnetosomes suspension containing 8 mg magnetosomes. After continuously stirred for 18 h at room temperature (25°C), the reactant was placed onto a magnet so that the magnetosomes were absorbed absolutely and the residual glutaraldehyde was got rid of by discarding supernatant. Then these magnetosomes and 4 mg monoclonal antibody was mixt and the pH value of this mixture was regulated to 9.5 by 1 M carbonic acid buffer. After incubated for 24 h at 4°C, the mixture was added to 0.1 ml 0.2 M lysine to occlude the residual aldehyde group and was absorbed by a magnet to get rid of dissociative antibody before it was conserved at 4°C as a spare.

### 1.2.5 The method of labeling with NaIO<sub>4</sub>

1 ml 0.06 M NaIO<sub>4</sub> solution was added into 1 ml magnetosomes suspension containing 8 mg magnetosomes and then mildly stirred for 2 h at temperature. To stop the oxidation reaction, the reactant was added 1 ml 0.16 M glycol into and mildly stirred at 4°C. The oxidized magnetosomes washed 4 times by 0.01 M carbonic acid buffer were added into 4 mg monoclonal antibody of HBsAg and the pH value of the reactant was adjusted to 9.5 before continuously stirred at room temperature for 2 h. After added 0.4 ml 0.1 M NaHB<sub>4</sub> into and incubated for 1 h at 4°C, the reactant was absorbed by a magnet to get rid of redundant antibody and NaHB<sub>4</sub> before it was conserved at 4°C as a spare.

### 1.2.6 The method of labeling with SPDP

1 µg SPDP was rapidly dissolved into 8 mg magnetosomes washed several times by 0.01 M PBS. After the reactant was mildly stirred for 30 min at 23°C, the magnetosomes were recovered by the way of magnet absorption and washed three times by 0.01 M PBS to get rid of products and the redundant SPDP. After added DTT solid into until the concentration of DTT was 25 mmol/L, the recovered magnetosomes were stirred frequently at 23°C for 25 min, and then were washed three times by 0.01 M PBS; The IgG of monoclonal antibody was treated by the same way of the magnetosomes except the concentration ratio of SPDP and IgG is 15 µg/mg. The mixture of the treated magnetosomes and the treated antibody was stirred frequently at 4°C for 20 h and washed by 0.01 M PBS.

## 1.3 Determination of the concentration of the labeled magnetosomes

The magnetosomes labeled by pure monoclonal antibody of HBsAg were washed three times and soaked into 0.01 M PBS containing 1% dried separated milk to block out. After incubated at 4°C for 24 h, the magnetosomes were washed by 0.01 M PBS and the redundant proteins were gotten rid of. The obtained pure monoclonal antibody, labeling magnetosomes, were diluted to a series of concentration and added into a white light tight polystyrene board. After AP labeled by polyclonal antibody was added into, the reactant was incubated at 37°C for 1 h and washed 5 times and then added DP into, measured by a luminescence instrument 10 min later.

## 1.4 The EDC method of labeling AP with polyclonal antibody of HbsAg

The purity of the polyclonal antibody purified by affinity chromatography was determined by SDS-PAGE. AP was labeled with polyclonal antibody of HBsAg by the way of EDC. 30 mg pure polyclonal antibody of HBsAg dissolved in 1.5 ml N, N-Dimethylformamide was added into 2 ml 10 mg/ml AP solution and then 10.8 mg EDC in all was added into in three batches that 3.6 mg EDC was added every hour in each batch. After stirred at 4°C for one night and dialyzed in 0.01 M Tris-HCl buffer (pH=7.0) to get rid of EDC absolutely, the mixture was added equi-volume glycerol into and conserved at -20°C.

### 1.5 Preparing for substrate solution of sensitivity-enhanced chemiluminescence reaction

After 600 µl ethanolamine, 100 µl 1 M NaOH, 100 µl 1 M MgCl<sub>2</sub> and 100 µl NaN<sub>3</sub>(10%) were commingled adequately, the buffer for substrate was prepared and sterilized by high-pressure sterilizer. Substrate solution was prepared by mixing 50 µl DP, 200 µl sensitivity-enhance reagent and 800 µl buffer for substrate in a sterilized vessel.

### 1.6 Detection of HBsAg labeling magnetosomes

The monoclonal antibody, labeling magnetosomes, were diluted to a series of concentration and added HBsAg standard samples into including sensitivity serum and specific serum. Then 50 µl serum desired to be detected and 50 µl work solution of polyclonal antibody of HBsAg labeled with AP were added into them. After being incubated at 37°C for 1 h, the reactant was washed 5 times with 0.01 M PBS (pH=7.4) containing 0.05% Tween-20 and added 100 µl chemiluminescence substrate solution into. The reactant, placed at room temperature for 10 min, was measured by Luminoskan Ascent.

## 2 Results

### 2.1 Labeling magnetosomes with antibody

2.16 µg proteins were coupled with 1 mg magnetosomes by the method of using SPDP, which was the highest among the 4 labeling methods including the method of direct absorption, labeling with glutaraldehyde, labeling with NaIO<sub>4</sub> and labeling with SPDP (see Tab. 1). So the method of labeling magnetosomes with SPDP was selected in the following experiments.

### 2.2 Measuring the sensitivity of the samples

The measure sensitivity threshold was defined as the

sum of the average value of 10 calibrating serum samples (containing no serum) and the double value of their standard deviation. The measure sensitivity of different labeling method was measured with a series of concentrations of sensitive serum of HBsAg as 0.1 ng/ml, 0.2 ng/ml, 0.5 ng/ml, 1 ng/ml. The sensitivity measured with the HBsAg that was treated with SPDP was highest and the result is 0.1 ng/ml (see Tab. 2).

**Tab. 1 Labeling efficiency of magnetosomes by different methods**

Labeling method	Labeling efficiency (µg proteins / mg magnetosomes)
Direct absorption	0.82
Treat with glutaraldehyde	1.26
Treat with NaIO <sub>4</sub>	0.32
Treat with SPDP	2.16

**Tab. 2 The measure sensitivity of different labeling method**

Sample	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	Threshold value
Direct absorption	0.024	0.034	0.093	0.121	0.100
Treat with glutaraldehyde	0.034	0.075	0.100	0.137	0.103
Treat with NaIO <sub>4</sub>	0.021	0.023	0.031	0.059	0.093
Treat with SPDP	0.102	0.378	0.531	0.892	0.100

### 2.3 Measuring specificity

As the results above showed, the labeling method with SPDP was best, thus was selected for measuring the specific serum. There were 20 negative samples and 3 positive samples among 23 specific serum samples provided as standard controls by National Institute for The Control of Pharmaceutical and Biological Products. The sensitivity results of magnetosomes reagent were accorded with the results of the known standard specific serum samples.

### 2.4 Comparing with ELISA

Comparing with the reagents of ELISA, the magneto-

somes reagent was used to detect 20 serum samples of hepatitis B patients, 30 serum samples of patients that carried hepatitis B virus and 50 random human serum samples. The results of the serum samples of hepatitis B patients and hepatitis B carriers, obtained with the magnetosomes reagent, were 100% consistent with those obtained with the reagents of ELISA. There were 7 positive samples among 50 random human serum samples when using the reagents of ELISA or the magnetosomes reagent, but there were other 2 positive samples when using the latter. The two people that were detected positive with the magnetosomes reagent but negative with the reagent of ELISA were tracking surveyed with the latter and one was detected positive 1 month later, the other was detected positive 2 month later (see Tab. 3). The reason of this phenomenon was that the threshold of sensitivity of the method of ELISA (0.5 ng/ml) was higher than that of the magnetosomes reagent (0.1 ng/ml), thus the antigens were not detected by the method of ELISA until the concentration of them was 0.5 ng/ml.

### 3 Discussion

The magnetosome membrane, similar to the cytoplasm membrane, contains lipids and a few proteins. Four methods for coupling the antibody to magnetosomes were designed in this experiment. The antibodies or the antigens were absorbed onto the surface of magnetosomes by the method of direct absorption, which lied on the hydrophobic interaction between the magnetosomes and the antibodies or antigens. The principle of the method of labeling with  $\text{NaIO}_4$  was that the Pinacol structure of the compound con-

taining glycosyl was oxidized by  $\text{NaIO}_4$  and became aldehyde group which reacted with the amino of proteins to form Schiff base. Glutaraldehyde, as a double function reagent, had two aldehyde groups which could react with two primamidos of two different or same molecules to form Schiff base and linked the two molecules with 5-carbon chains. Result indicated that the magnetosomes coupling efficiency of the methods above were not high and the react condition need to deeply groped for. The active lipid components of SPDP could react with the amidos of magnetosome membrane and the 2-pyridine-disulfide of SPDP could react with fatty acid thiol. Thus SPDP could introduce sulfhydryl into molecule of protein and link with other molecule. This method could link many products which were steady and not easy to self-aggregate.

Combined with chemiluminescence-immunoassays, HBsAgs in human body were quantitatively detected by using magnetosomes as carriers of monoclonal antibody and the sensitivity threshold of this method was 0.1 ng/ml, which was 5 times of the method of ELISA. The amounts of antibody coupled with 1 mg magnetosomes by SPDP in this study was only 2.16  $\mu\text{g}$ , which was not large and possibly related with the coupling conditions and the purity of magnetosomes. Thus it is the emphasis of the following study to grope for coupling conditions and enhance the couple efficiency of magnetosomes and antibody. Now, a simple approach for rapid extraction and purification of magnetosomes was established. The method for detecting the purity of the magnetosomes by electron microscopy, energy spectrometer and infrared spectrometric analyzer was established and the standard for evaluating the quality

**Tab. 3 the results of detecting HBsAg in human body by magnetosomes reagent or the ELISA reagent**

The source of samples	Number	ELISA		Chemiluminescence		Consistency
		Positive	Negative	Positive	Negative	
Hepatitis B patients	20	20	0	20	0	100%
Hepatitis B virus carriers	30	30	0	30	0	100%
Random people	50	7+2*	43	9	41	100%

Note: \* tracking surveyed results

of magnetosomes by infra-red sepectrometry was presented at the same time (data not shown). Using magnetosomes for detecting HBsAg was unreported so far. Data of this study showed that it was completely feasible to detect HBsAg at the early infection stage. It is expected that this kind of nano-biomaterial possessing steady crystal morphology could be used to service human as early as possible.

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