

Use of biosensor as a tool for double antibody sandwich immunoassay establishment: Screening of appropriate matched monoclonal antibody pair

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ABSTRACT

Background: For biomarker detection, double antibody sandwich platform is the most sensitive and specific immunoassay. Development of double antibody sandwich platform requires the step of appropriate antibody pair selection. However, identification of matched antibody pairs is difficult, laborious and time-consuming.

Objectives: To illustrate the application of biosensor for selecting the suitable matched pairs of antibody for double antibody sandwich platform.

Materials and methods: Double antibody sandwich immunoassay for Ag85B protein detection was selected as a model study. Biolayer interferometry biosensor was used to search matched pairs of antibody for Ag85B protein detection. To prove the obtained information, the double antibody sandwich ELISAs were employed.

Results: Biosensor results revealed that the best-matched antibody pair might be mAb clone AM85B-8B as capture antibody and mAb clone AM85B-5B or AM85B-9B as detecting antibody. The results obtained from biosensor system and ELISA were similar.

Conclusion: Biosensor can be used as a tool for effectively screening and selecting the best pairs of antibodies for any immunoassays. Biosensor method is very useful for reducing processes and time for double antibody immunoassay development.

Introduction

Nowadays, immunoassays are widely used in several fields including clinical, pharmaceutical, environmental and food analysis.¹⁻⁵ Immunoassays are based on the principle of antigen-antibody reactions. Specificity and selectivity of the antibodies used lead to many applications in detection and quantification of biological molecules of interest.

Immunoassay design for detection of any antigens can be classified into two broad categories: 1) label-immunoassay; 2) non-label immunoassay; which depend on the use of antibody or antigen labeling with radioisotopes, enzymes, fluorescence dyes and luminescent substances or non-labelling, respectively.⁶

For detection and measurement of antigen of interest, the most sensitive immunoassay design is double antibody sandwich immunoassays using two specific antibodies.⁷ By this technique, the antigens to be measured are captured onto specific antibody-coated solid phase (capture antibody), samples are cleaned up and antigens are enriched. Subsequently, the captured antigens are allowed to react with a labeled secondary specific antibody

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(detection antibody). As a result, the antigen is 'sandwiched' between the two antibodies, the capture and detection antibodies. Amount of antigen measured is correlated with the amount of the labeled secondary antibody bound to the solid phase. Subsequently, antigen concentration is directly proportional to the intensity of the detecting signal.^{6,8}

Antibodies used in immunoassays for the detection of the molecule of interest can be either monoclonal or polyclonal. The efficacy of an immunoassay depends upon the properties of antibody such as specific recognition, affinity and avidity.⁹ Importantly, the capture and detection antibodies must recognize two non-overlapping epitopes on the antigen, must not interfere with one another and must be able to bind to the antigen simultaneously.^{8, 10} In immunoassay development for detection of molecules of interest, selection of appropriate antibodies is, therefore, one of the critical steps.¹¹

In general, checkerboard assays using microplates are appropriate for the screening of appropriate antibody pairs.¹¹⁻¹³ However, identification of matched antibody pairs is difficult or cannot be determined in the case of the use of antibody pairs derived from one animal species.¹¹ Matched pair screening step requires a large amount of purified and labeled antibodies. These processes are laborious, time consuming and expensive.¹¹⁻¹³ Accordingly, new methods for effectively screening and selecting the best pairs of antibodies for any immunoassays are needed.¹⁴ In this study, we introduced a new method for the aforementioned purpose. We demonstrated the use of biolayer interferometry biosensor can be applied for selecting the best matched pairs of antibody. The described strategy can be used to determine the antibodies that should be used as the capture antibody and which antibody should be used as the detection antibody. The described method is very useful for reducing processes and time for immunoassay development.

Materials and methods

Antibodies and reagents

The anti-Ag85B monoclonal antibody (mAb) clones AM85B-5B (IgG2b), AM85B-8B (IgG1) and AM85B-9B (IgG1) were produced in our laboratory.¹⁵ The recombinant Ag85B protein linked with biotin carboxyl carrier protein (Ag85B-BCCP) was generated in our laboratory.¹⁵ EZ-Link™ Sulfo-NHS-Biotin was obtained from Pierce (Rockford, IL, USA). The BLItz biolayer interferometry biosensor and the streptavidin sensor chips were purchased from FortéBio (Pall Life Sciences, Menlo Park, CA, USA).

Biotinylation of antibodies and generation of double antibody platform using biosensor system

All clones of antibodies were biotinylated using EZ-Link™ Sulfo-NHS-LC-Biotin according to manufacturer instructions.¹⁶ Each biotin labeled antibody was used as capture antibody and immobilized onto streptavidin sensor chips. The sensor chips were placed into sample diluent buffer (PBS containing 0.1% bovine serum albumin, 0.02% Tween-20, 0.05% Na₂S₂O₃).

The biotinylated anti-Ag85B mAb sensors were immersed into Ag85B-BCCP or CD147 BCCP (as control). The sensor tips were placed into sample diluent buffer. Then, anti-Ag85B mAb sensors were immersed into the non-biotinylated mAbs (detecting mAbs). The binding signal was measured using a BLItz biolayer interferometry biosensor and calculated as delta binding signal, which is obtained by subtracting the binding signal at the end point of the washing step from the end point of non-biotinylated detecting antibody adding step.

Detection of Ag85B-BCCP by ELISA

The capture antibody against Ag85B was coated on ELISA plate. The recombinant Ag85B-BCCP proteins were added and incubated at 37 °C for 1 hour. After washing, the biotinylated mAbs, which recognizes different epitopes from the capture mAbs to Ag85B-BCCP proteins, were added into plate and followed by HRP-conjugated streptavidin. The reactions were detected using a TMB substrate (Zymed) and optical density was measured at 450 nm.

Results

Methods for selecting an appropriate pair of antibodies using an immunoassay for detecting molecules of interest are always complicated. In this study, double antibody biosensor was designed (Figure 1A) for selecting the appropriate pair of mAbs for the development of any type of immunoassay. Capture antibody was labeled with biotin and immobilized on a streptavidin biosensor, then antigen was added and bound with the immobilized capture mAbs on the sensor chip. Subsequently, the sensor was immersed into non-biotinylated specific antibody (detecting mAb). The binding signals at every steps were measured using a BLItz biolayer interferometry biosensor as displayed in sensogram (Figure 1B).

In this study, recombinant proteins Ag85B (*Mycobacterium tuberculosis* antigen), were used as study model. For Ag85B antigen detection system, three capture mAbs (anti-Ag85B mAbs clones AM85B-5B, AM85B-8B and AM85B-9B) were tested in order to obtain the best pair of mAbs in sandwich immunoassay which showed the highest detection signal when using different clones of detecting mAbs. Binding signal obtained from biotinylated anti-Ag85B capture mAb clone AM85B-8B showed the strongest binding signal when using either detecting mAb clone AM85B-5B or AM85B-9B (Figure 2). However, the best matched pair of mAbs that should be used in further development of sandwich immunoassay for detecting Ag85B proteins was using anti-Ag85B mAb clone AM85B-8B mAb as capture antibody and anti-Ag85B mAb clone AM85B-9B as detection antibody (Figure 2).

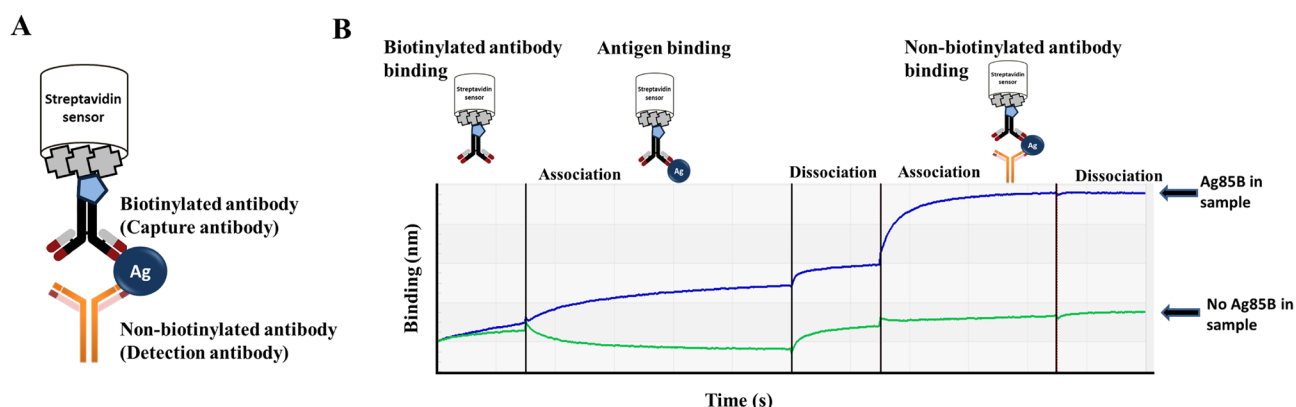


Figure 1. Schematic illustration of double antibody biosensor platform. (A) The model of double antibody sandwich biosensor design. (B) The sensogram demonstrates the binding signal obtained from the BLItz biolayer interferometry biosensor at the indicated step.

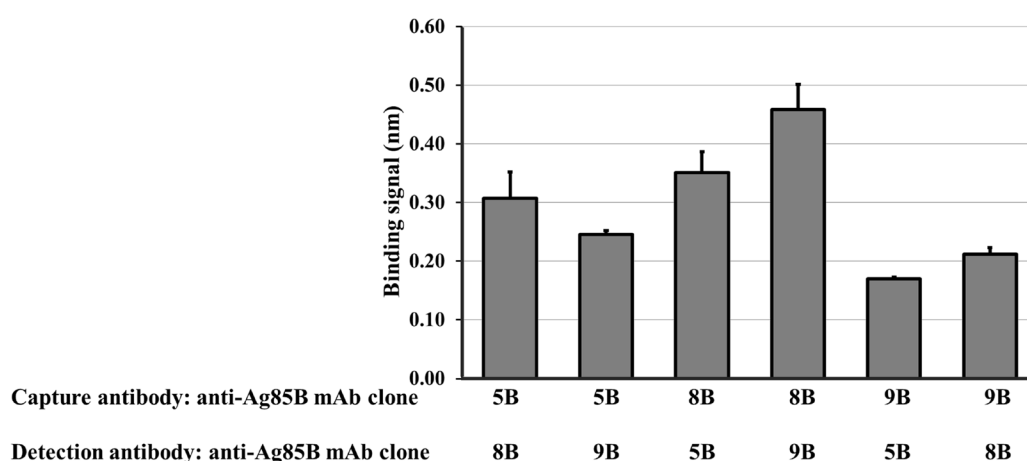


Figure 2. Detection of specific antigen using double antibody sandwich biosensor. The capture antibodies as indicated were immobilized onto streptavidin sensor chip. The specific antigens were added and followed by the detection antibodies as indicated. The detection of Ag85B was plotted as delta binding signal. The bar graph shows mean $\pm$ SD of three independent experiments.

To confirm the finding of appropriate pair of mAbs by biosensor, we further performed the experiment by using double antibody sandwich ELISA which is the common immunoassay available as a commercial product. As shown in Figure 3, the use of anti-Ag85B mAb clone Ag85B-8B as capture antibody showed the highest absorbance signal, followed by clone 5B and clone 9B, respectively. This obtained data was similar to that using biosensor system. The result illustrated that, in agreement with the biosensor

platform, anti-Ag85B mAb clone AM85B-8B as capture antibody and anti-Ag85B clone AM85B-9B as detection antibody was the appropriate mAb pair in using sandwich immunoassay for detection of Ag85B proteins.

Taken together, our study suggested that the biosensor system can be used as a simple and rapid screening method to obtain the pair of antibody for development of immunoassay.

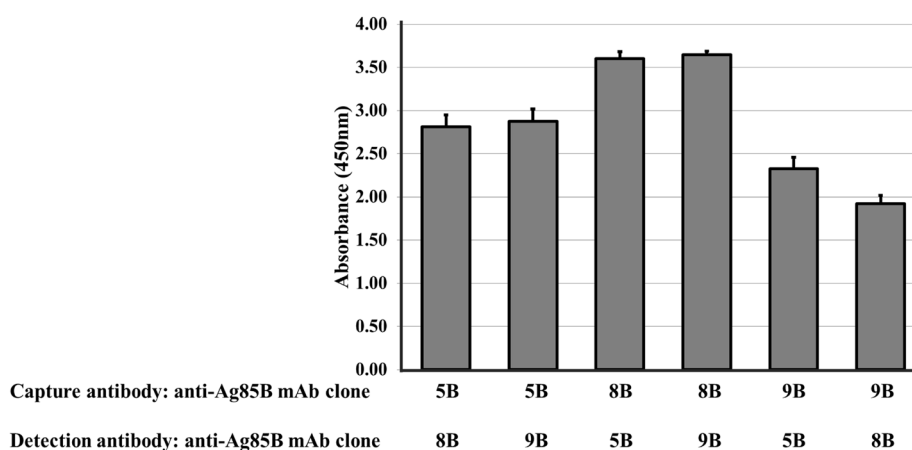


Figure 3. Detection of specific antigen using double antibody sandwich ELISA. The capture antibodies as illustrated were coated onto ELISA plate. The specific antigens were added into each well. The detection antibodies, which bind to different epitope, were added and followed by conjugates and color substrate. The optical intensity for measurement of Ag85B is shown as mean $\pm$ SD of duplicate experiments.

Discussion

Since an antibody binds specifically to its antigen, immunoassay becomes a popular strategy to detect the presence or absence of substances of interest such as biomarkers and drugs.^{3, 4, 15, 17} Although the commercial immunoassays are available in different formats, ie. ELISA, immunochromatographic strip test, beads-based immunoassay^{18, 19} all of the commercial immunoassay require the same steps during the set-up process including design of assay platform, selection of appropriate antibodies, optimization and validation.^{8, 13, 17} Step of immunoassay development is time consuming; therefore, shortening time in each development process is a key point for the commercial development of diagnostic immunoassay.¹⁴

To develop immunoassay for antigen measurement, double antibody sandwich platform is usually designed according to the sensitivity and specificity.^{2, 7, 12} Performance of sandwich immunoassay is affected by several factors especially characteristic of the antibody used in the assay. Selection of high affinity suitable pairs of antibodies is an important step of immunoassay development. Selection of the pair of antigen or antibody for use in assay is commonly based on ELISA which is laborious, requires a well-trained person and need a long incubation period.^{11, 19} Moreover, purified specific antibodies are required for both capture and detection antibodies and pairs derived from one animal species are desired. The mAb purification step is also time-consuming, labor intensive and costly. As a consequence, there has been much interest in the development of a speedy, simple and cost-effective method for matched pairs monoclonal antibody identification that reduces the use of purified mAbs.¹⁴

Thus, in this study, the alternative approach for screening pairs of antibodies is introduced which is biolayer interferometry biosensor. This system is based on the principle of the optical interferometry called the interaction of light waves. Device detects the wavelength shift between before and after the biomolecule or antigen binds to its ligand or antibody. Then, the delta binding is

real-time generated and plotted as binding signal which reflects the amount of detected antigen or antibody. Using this property, the binding characteristic of capture and detection antibodies with antigen in terms of overlapping epitope, interference and influence are revealed within 5 minutes. Therefore, it is fast and easy to discover the properly matched antibody pair. In addition, the kinetic rate of antibody-antigen binding can be obtained including association rate (K_a), dissociation rate (k_d) and affinity constant (KD ; K_a/K_d) by the biolayer interferometry biosensor.²⁰⁻²² By the biosensor system, binding signal of mAb and antigen could be determined in a real-time manner. Association rate of antigen binding signal can be visualized and the high association rate takes a shorter time to reach the highest stable binding signal whereas the low association rate appears over a longer time. Dissociation rate can be observed in the washing step after antigen binding, when the high affinity antibody shows a stable binding signal. In contrast, the low affinity antibody illustrates a rapidly decreasing binding signal. Notably, the association and dissociation rate of antigen and antibody binding can be visualized and obtained from the information in the software. As a result, the suitable capture and detection antibodies are identified. In addition, the biosensor system requires a small volume and amount of antibody: only 4 ng of antibody is enough to use in the assay.²⁰⁻²²

In order to prove our concept, several clones of the monoclonal antibody against Ag85B and recombinant protein Ag85B were used as model of the study. Hence, labeling one of the antibodies with biotin or enzyme is required and then it is matched with a non-label antibody.^{11, 12} In this study, antibodies were labeled with biotin which is a small biomolecule and does not interfere with the binding site of antibodies. Moreover, the reaction of biotin and streptavidin is a strong non-covalent reaction resulting in a tight binding between the biotinylated antibody and either streptavidin sensor chips or streptavidin-enzyme conjugate.²³

The results obtained from biosensor system and

ELISA were comparable where the appropriate pair of antibody could be selected. Using biosensor system showed the benefit over ELISA method in several tasks. The incubation time could be reduced from at least 4 hours (ELISA) to 7 minutes (biosensor). In addition, the amount of antibody and antigen used in biosensor system required only 4 μ l in the individual step, which is lower than the amount of antigen or antibody used in ELISA (50 μ l). Therefore, the scale of antigen or antibody production for the step of antibody screening could be minimized and translated to minimizing cost, time for antibody production and labeling. In addition, incubation time of biosensor system is less than ELISA. The early step in immunoassay development, screening pairs of antibodies, would be faster and could move forward the step of assay set up. Hence, the double antibody biosensor platform might be used as the screening system to select the appropriate pair of mAb for further immunoassay development.

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Conflict of interest

The authors declare that they have no competing interests.

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