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Strategies for the optimization of bead-immunoassays for the effective detection of target biomolecules

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Abstract−Immunoassays are analytical methods using antibody-specific reactions to analyze samples. Due to recent developments in antibody technology, the scope of potential samples has expanded to not only proteins, but also low molecular-weight compounds, carbohydrates, lipids, and microorganisms. Immunoassays have the advantage of being highly sensitive, capable of detecting small amounts, and thus have potential for application in biosensors. Immunoassays using magnetic beads have been developed and can be converted to more diverse platforms than the existing limited well plate-based assay. Furthermore, magnetic bead immunoassays detect analytical samples more quickly, and are becoming one of the most suitable immunoassay tools applicable to biosensors. However, their development requires optimization for the improvement of detection ability for specific samples. Therefore, we propose a guideline for solving detection problems occurring in magnetic bead immunoassay optimization processes. It is aimed to be a good reference, enabling researchers performing such optimization more quickly and efficiently.

Keywords: Immunoassay, Magnetic Bead, Assay Optimization, Colorimetric Assay, Biosensor

INTRODUCTION

Immunoassays involve the use of antibodies to analyze a sample, and are a method of detecting or quantifying a sample using a specific binding reactions of an antibody to the target, such as a protein [1]. Immunoassays were developed in the 1960s, starting with radioimmunoassays for protein analysis. The enzyme-linked immunosorbent assay (ELISA) was developed in 1971 and a fluorescence-based detection method using a fluorescent substrate was developed in 1979 [1,2]. Immunoassays include radioimmunoassays (RIAs) for detecting signals using radioactive isotopes, ELISAs using signal amplification through enzymes, enzyme immunoassays (EIAs) employing fluorescent antibodies as a reporter, and chemiluminescence immunoassays (CLIA) [3]. Originally, researchers mainly used immunoassays for protein detection and quantification. Recently, antibody technology has been used to analyze low molecular weight compounds, carbohydrates, lipids, and various microorganisms [2]. Over the last 50 years, remarkable progress has been made in improving the detection sensitivity and multi-analyzing ability of immunoassays, and this development has naturally been applied to the development of biosensors based on immunoassay methods. In principle, the basis of all immunoassays is the process of immobilizing the antigen or antibody to be analyzed on a specific solid surface. Since immunoassays were developed, they have been performed using a method of immobilizing specific antigens or antibodies on the surface of a well plate, such as a 96-well plate [4]. However, this conventional method has limitations, as biosensors require various platforms. In addition, detection and quantification of a sample through a well plate immunoassay, after immobilization of the antigen or antibody, requires a long time to process and therefore to detect the final sample. Consequently, this detection technique is a clear limitation in the development of a biosensor requiring fast and rapid detection [5]. To overcome these limitations, immunoassays using magnetic beads have emerged as a new platform to develop immunoassay-based biosensors [6,7]. The approach of using magnetic beads was developed by Gundersen in 1991, by conjugating an antibody to a magnetic bead surface, reacting them with specific antigens, and then separating them using magnets [8,9]. As a result, it was possible to detect samples with a more simple and efficient experimental procedure [7,10]. Another advantage of the bead assay is that it can be modified into various formulations, according to target particle sizes. As a result, the immunoassay method using magnetic beads has been developed steadily in recent years [7,11-13]. However, these assays still require an optimization process to improve their detection limit and sensitivity for a specific sample. In contrast to the conventional immunoassay using a well plate, there are many more variables to consider in this optimization process [14-16]. Therefore, we propose a formal approach for analyzing and solving the various experimental problems involved in the optimization process for an immunoassay-based biosensor using magnetic beads. It is often difficult for researchers first performing magnetic bead immunoassay optimization to know what problems are likely, and how their results may differ from the expected results. There are many cases where it is necessary to think about strategies to solve such problems. This paper therefore provides a guideline for

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the interpretation of typical problems that may occur in the optimization process of bead assays, and suggests solution strategies to solve these problems quickly. From this, researchers performing magnetic bead immunoassays can optimize the process more quickly and effectively.

MATERIALS AND METHODS

1. Materials

Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Carboxylated magnetic bead (Dynabeads M-270 carboxyl acid; 2×10^9 beads/mL; approximately 30 mg/mL) were purchased from Thermo Fisher Scientific (Massachusetts, USA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant human SMPD1 proteins, goat polyclonal IgG antibodies against human SMPD1, and mouse monoclonal IgG antibodies against human SMPD1 were obtained from R&D systems (Minnesota, USA). Anti-goat IgG - and Anti-mouse IgG - peroxidase antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alexa Fluor 488 antibody labeling kits were acquired from Thermo Fisher Scientific (Massachusetts, USA). Buffers 2-(N-morpholino)ethanesulfonic acid (MES) and PBS were used at pH 5 and 7.4, respectively, unless otherwise stated.

2. Comparison of Optical Density Background Level with Different Secondary Antibody Ratio

Magnetic beads (50 μL; 30 mg/mL) were washed in 50 μL 25 mM MES buffer for 10 min with thorough mixing. After centrifugation, the tube was placed on a magnet for 4 min and the supernatant was removed. To this, 50 μL of EDC solution dissolved in 25 mM MES buffer and 50 μL of NHS solution dissolved in 25 mM MES buffer, was added. The solution was mixed well and incubated with slow tilt rotation at room temperature for 30 min. After incubation, the tube was placed on the magnet, the supernatant was removed, and the tube was washed three times with 100 μL 25 mM MES buffer. Next, 150 μL of 1 μg/mL human SMPD1 mouse monoclonal IgG antibody in 25 mM MES buffer was added to the washed beads for conjugation. This solution was incubated for 30 min with slow tilt rotation at room temperature. After incubation, the tube was placed on the magnet, the supernatant removed, then washed three times with 100 μL of 25 mM MES buffer. To compare background absorbance levels at different secondary antibody ratios, without analyte proteins, 150 μL of 2 μg/mL human SMPD1 goat IgG polyclonal antibody in 1 x PBS was directly added to the monoclonal antibody conjugated beads and incubated with slow tilt rotation at room temperature for 2 h. After incubation, the tube was placed on the magnet, the supernatant was removed, and the tube was washed three times with 100 μL 25 mM MES buffer. Next, 200 μL of anti-goat IgG-peroxidase antibodies (at various dilution ratios) in 1 x PBS buffer was added to the washed beads and incubated with slow tilt rotation for 1.5 h. After incubation, the tube was placed on the magnet and the supernatant removed. Next, 200 μL 2,2′-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS, a peroxidase substrate) solution was added to the beads and incubated for 15 min. The tube was placed on a magnet and supernatant then transferred to a 96 well microplate. Absorbance at 450 nm was measured with a microplate reader (Biotek, VT, USA).

3. Comparison of Optical Density Sensitivity with Different Blocking Methods

3-1. Comparison of Optical Density Sensitivity with Different Bead Surface Coating or Chemical Quenching Procedures

Magnetic beads were prepared as above (section 2.2) until the primary antibody incubation. To compare the background absorbance level with different blocking methods, 2% bovine serum albumin (BSA) solution in 25 mM MES buffer, 5% BSA solution in 25 mM MES buffer, 5% skim milk solution in 25 mM MES buffer, and 50 mM ethanolamine in 1 x PBS, pH 8.0 were added to the washed beads. After incubation, the tube was placed on the magnet, the supernatant was removed, then the tube was washed three times with 1 x PBS buffer. After blocking with the various agents, secondary antibody staining and visualization was carried out as above (Section 2.2).

3-2. Identification of Absorbance Level with Chemical Quenching Blocking Method

After determining that the chemical quenching method had better blocking efficiency than the bead surface coating method, we measured the absorbance level in the presence of analyte proteins with the chemical quenching blocking step, as these chemical reactions have a potential to damage the analytes or antibodies. Magnetic beads were prepared as above (Section 2.2). Next, 150 μL of 440 ng/mL human SMPD1 protein solution in 25 mM MES buffer was added to the washed beads, and incubated for 60 min with slow tilt rotation at room temperature. After incubation, the tube was placed on the magnet, the supernatant removed, then washed three times with 100 μL 25 mM MES buffer. Washed beads were chemically quenched by adding 150 μL of 50 mM ethanol amine in 1 x PBS, pH 8.0, then incubated for 60 min with slow tilt rotation. After incubation, the tube was placed on a magnet, the supernatant removed, then washed three times with 150 μL of 1 x PBS. Either 150 μL of 2 μg/mL human SMPD1 mouse monoclonal antibodies in 1 x PBS or 150 μL of 2 μg/mL human SMPD1 goat polyclonal antibodies in 1 x PBS was added to the tube and incubated for 2 h with slow tilt rotator at room temperature. After incubation, the tube was placed on a magnet, the supernatant removed, then washed three times with 150 μL of 1 x PBS. Next either 200 μL of anti-mouse IgG-peroxidase antibodies (1 : 15,000, diluted in 1 x PBS) or 200 μL of anti-goat IgG-peroxidase antibodies (1 : 15,000 diluted in 1 x PBS) was added to the washed beads and incubated for 1.5 h. After incubation, samples were treated with peroxidase, substrate, and quantified as above (section 2.2).

4. Comparison of Optical Density Sensitivity with Different Colorimetric Methods

4-1. Magnetic Bead Immunoassay with Fluorescent Dye-labeled Secondary Antibodies

Fluorescent dye-labeled human SMPD1 polyclonal antibodies were prepared with Apex Alexa Fluor 488 antibody labeling kits (A10468; Thermo Fisher Scientific). Beads were washed, prepared and coated with primary antibodies as above (Section 2.2.). Next, 600 μL of each concentration of human SMPD1 protein solution in 1 x PBS, was added to each tube and incubated with slow tilt rota-

tion at room temperature for 2 h. After incubation, the tube was placed on a magnet, the supernatant removed, then samples washed three times with 150 μL of 1 x PBS. Next, 300 μL of 2 μg/mL Alexa 488 dye labeled human SMPD1 goat polyclonal antibodies in 1 x PBS was added to the washed beads, then incubated for 2 h in the dark with slow tilt rotation at room temperature. After incubation, the tube was placed on a magnet, the supernatant removed, then samples washed three times with 150 μL of 1 x PBS. Beads were resuspended in 300 μL of 1 x PBS. The solutions were then transferred into a 96 well plate and fluorescence at 490 nm measured.

4-2. Magnetic Bead Immunoassay with Horse Radish Peroxidase Labeled Secondary Antibodies

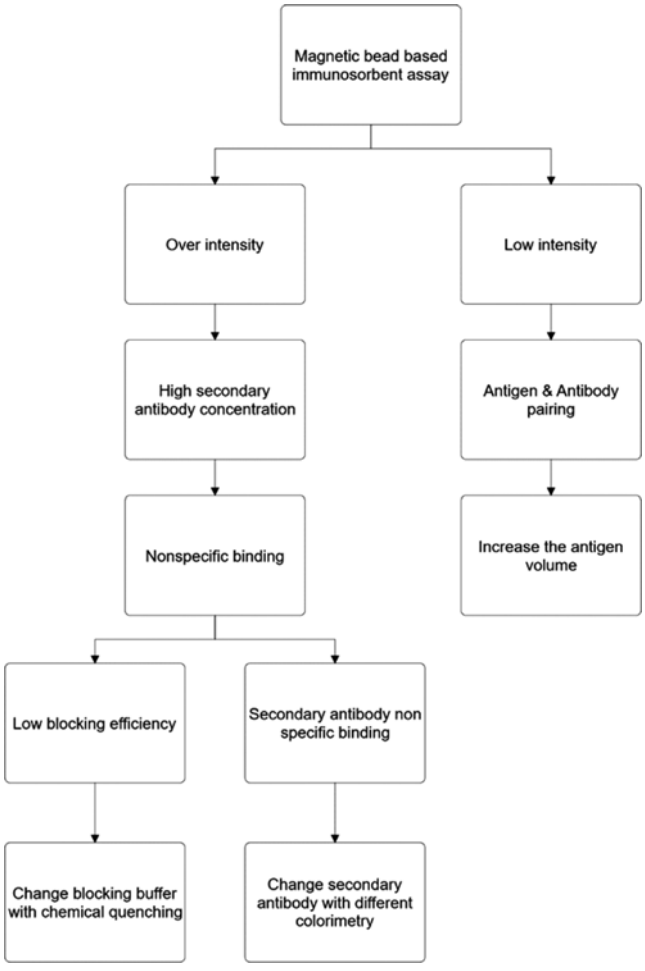
Beads were washed, prepared and coated with primary antibodies as above (Section 2.2.). To eliminate non-specific binding, 150 μL of 50 mM ethanol amine in 1 x PBS, pH 8.0 was added to the washed beads and tubes were incubated for 60 min with slow tilt rotation. After incubation, the tube was placed on a magnet, the supernatant removed, then samples washed three times with 150 μL of 1 x PBS. Next, 600 μL of each concentration of human SMPD1 protein solution in 1 x PBS, was added to each tube and incubated with slow tilt rotation at room temperature for 2 h. After incubation, the tube was placed on a magnet, the supernatant removed, then samples washed three times with 150 μL of 1 x PBS. Next, 300 μL of 2 μg/mL human SMPD1 goat polyclonal antibodies in 1 x PBS was added to the washed beads and incubated for 2 h with slow tilt rotation at room temperature. After incubation, the tube was placed on a magnet, the supernatant removed, then samples washed three times with 150 μL of 1 x PBS. Then 200 μL of anti-goat IgG-peroxidase antibodies (1 : 15,000, diluted in 1 x PBS) was added to the washed beads and incubated for 1.5 h. ABTS substrate was added and samples were quantified as above (section 2.2).

5. Comparison of Optical Density Sensitivity with Different Analyte Volumes

Beads were prepared as above (section 2.2.), with chemical quenching (as in section 2.3.1). To compare optical density sensitivity of different analyte volumes with the same concentration, either 150 μL or 600 μL of the same concentration human SMPD1 protein solution was added to each tube. After incubation for 2 h with slow tilt rotation, the tube was placed on the magnet for 4 min and the supernatant was removed. Beads were washed three times with 1 x PBS, then 300 μL of 2 μg/mL human SMPD1 goat polyclonal antibodies in 1 x PBS was added and incubated for 2 h with slow tilt rotation at room temperature. After incubation, the tube was placed on a magnet, the supernatant removed, then samples washed three times with 150 μL of 1 x PBS. Then 200 μL of anti-goat IgG-peroxidase antibodies (1 : 15,000, diluted in 1 x PBS) was added to the washed beads and incubated for 1.5 h. Substrate was added, and samples were processed, then quantified as described above (Section 2.2).

RESULTS AND DISCUSSION

When overintensity is generated in the magnetic bead-based immunosorbent assay, the first problem-shooting strategy is to reduce the concentration of the secondary antibody, which is the



Scheme 1. Flow chart for the troubleshooting of the bead assay optimization process.

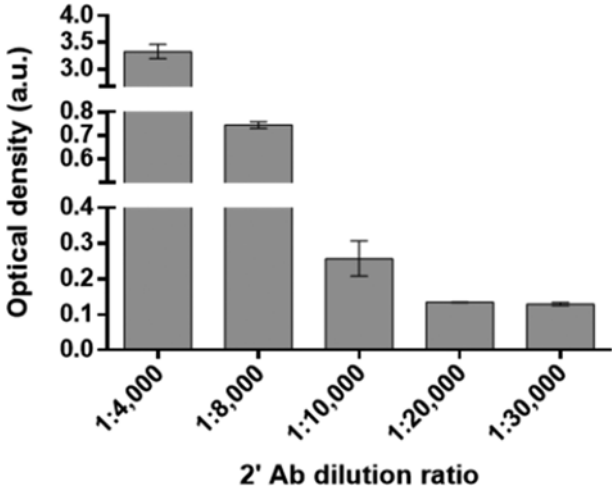


Fig. 1. The decreasing tendency of background optical density in magnetic bead immunoassay with changes in concentration of secondary antibody. Comparison of absorbance according to HRP - linked detection antibody dilution ratio during magnetic bead assay of sandwich ELSIA at 0 pg/mL target protein concentration.

detecting antibody. When the concentration of the secondary antibody is high, the detection signal is increased by residual detecting antibody remaining after the washing process and non-specific binding; therefore, a signal due to the antibody bound to the actual target agent is not detected. It is necessary to first check whether there is a difference in intensity between the control and sample groups, with the overall detection intensity lowered by reducing the concentration of the secondary antibody. As shown in Fig. 1, when overintensity occurs, the overall intensity is lowered by reducing the concentration of the secondary antibody. In spite of this, when there is no difference in the intensity between the control and sample group, blocking efficiency is low and the non-specific binding of detection antibody is increased. This is because the intensity of the detection antibody binding to a small amount of target molecule is less than that of the background signal. This may result in no difference in intensity between control and sample group.

Fig. 2(a) shows the result of coating a solid phase region, except the target antibody or sandwich ELISA primary antibody, with a molecule such as bovine serum albumin or skimmed milk, both widely used blocking method in ELISA. Using this method, the non-specific binding of the detecting antibody was reduced in the magnetic bead assay, but still shows a high intensity. In the case of magnetic beads, coating methods using specific biomolecules are limited and other blocking methods should be considered.

Therefore, a better blocking efficiency can be observed by using a new blocking method that eliminates the possibility of binding to a magnetic bead surface, using a chemical reaction such as that shown in Scheme 2(b), as compared to the conventional blocking method such as in Scheme 2(a). In Fig. 2(b), the antigen was conjugated to a magnetic bead surface using a direct ELISA method, and then the region (except the antigen binding site) was blocked using a chemical reaction. When the assay was performed using the monoclonal antibody and the polyclonal antibody for each of the enzyme-linked target factors, a high intensity difference was observed in the sample group compared to the control. From this,

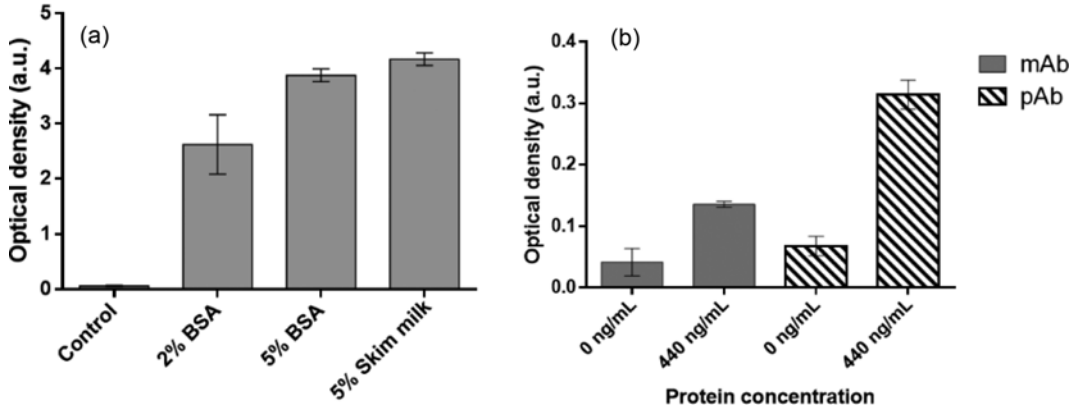
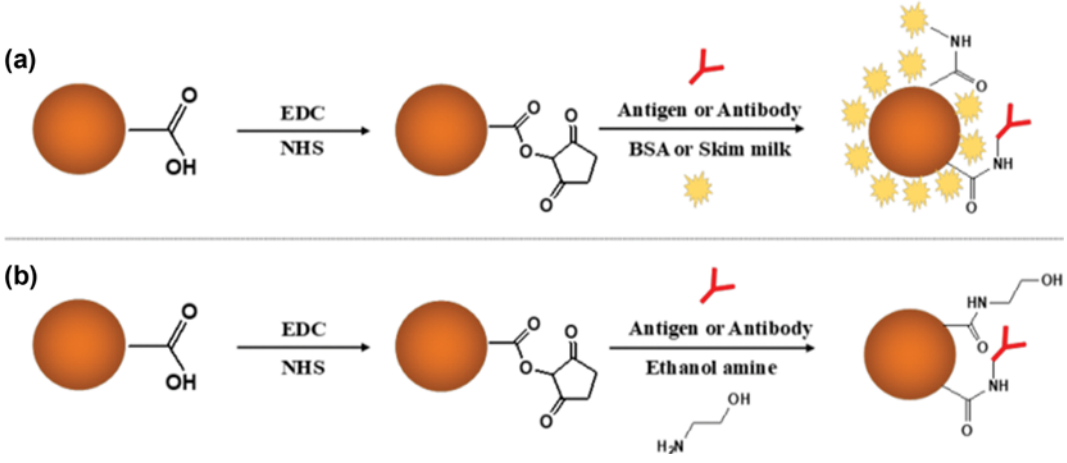


Fig. 2. Absorbance sensitivity according to magnetic bead immunoassay blocking method. (a) Absorbance comparison at a target concentration of 0 pg/mL under magnetic bead assay using the molecular coating blocking methods of bovine serum albumin or skim milk. The control is the absorbance in the chemical reaction blocking method using ethanol amine. (b) Comparison of detecting value of HRP-labeled anti-SMPD1 monoclonal antibody (mAb) and HRP-labeled anti-SMPD1 polyclonal antibody (pAb) against target protein SMPD1 conjugated to bead surface by direct ELISA using the chemical reaction blocking method using ethanol amine.



Scheme 2. A schematic diagram of the blocking methods of the magnetic bead immunoassay. (a) Bead surface coating blocking method using BSA or skim milk. (b) Magnetic bead immunoassay blocking method using the chemical quenching reaction.

the benefit of using a blocking method using a chemical reaction was shown.

Following the strategy of changing the blocking method using a chemical reaction, the second strategy to achieve a more sensitive intensity difference between the control and sample groups was changing the colorimetry method of the detecting antibody. This may be due to the very weak intensity emitted by the detecting antibody bound at low target concentration. Therefore, if a different colorimetry method can be used to amplify the intensity that one detecting antibody can emit, a meaningful detecting value can be obtained even under a very small target concentration.

Fig. 3(a) shows a comparison of the intensity differences in the control sample versus the control when the magnetic bead assay was performed using a sandwich ELISA method, using a detection antibody labeled with a fluorescence dye as in Scheme 3(a). Fig. 3(b) compares the intensity differences of the magnetic bead assay using the horseradish peroxidase-linked detection antibody, as in Scheme, 3(b) under the same conditions as in Fig. 3(a). In Fig. 3(a), there is no difference in intensity of the target protein concentration of 44 pg/mL and 440 pg/mL compared to control. Only when the target concentration was increased to 440 ng/mL was there a significant difference in intensity compared to the control. On the other hand, in the case of the assay using the enzyme-linked detection antibody of Fig. 3(b), a significant difference in intensity was observed even at a very sensitive concentration of 44 pg/mL. Therefore, when the magnetic bead assay shows poor sensitivity, it can be redesigned to have detectable sensitivity by changing the colorimetry of the detecting antibody.

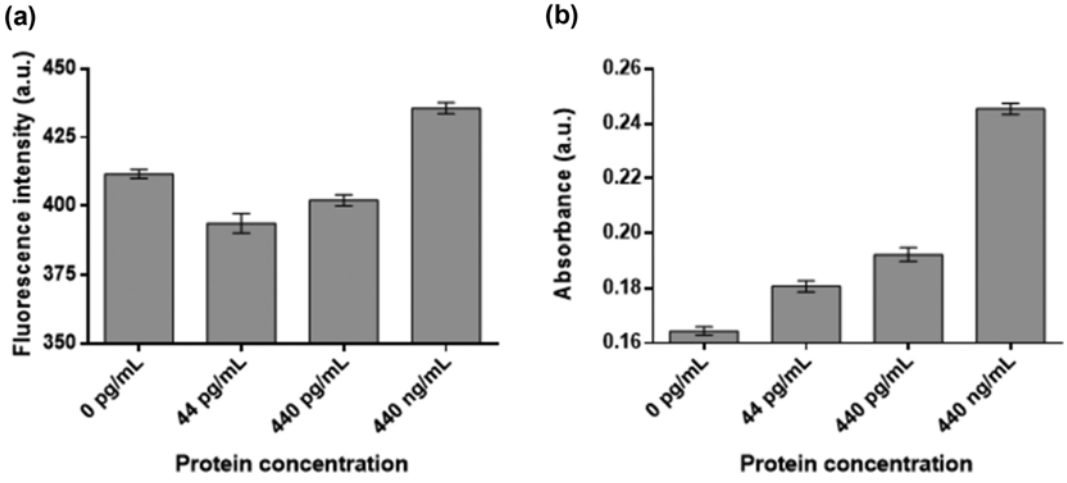
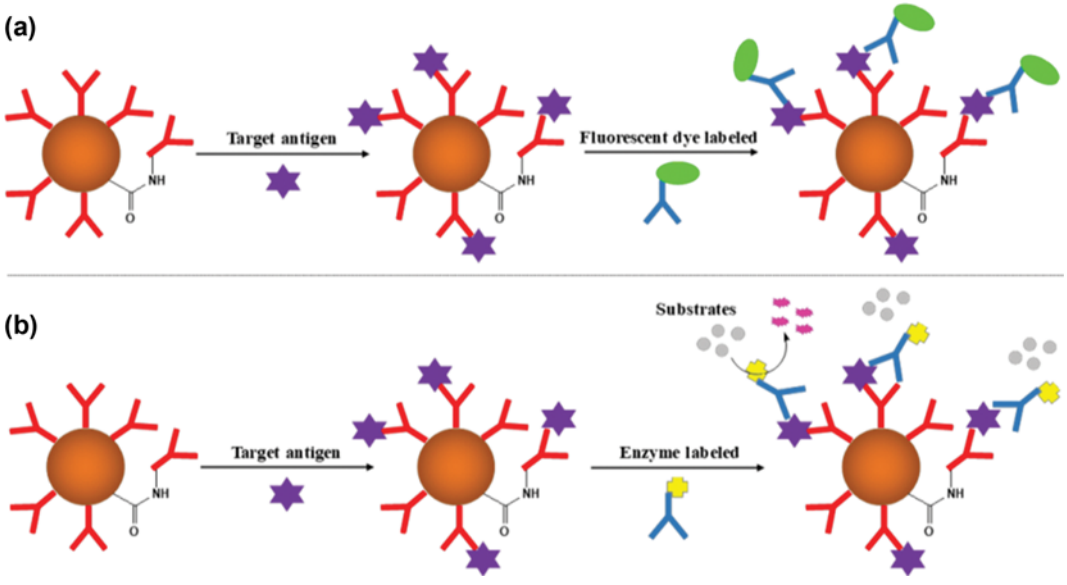


Fig. 3. Comparison of the magnetic bead immunoassay sensitivity with different colorimetric methods. (a) A magnetic bead immunoassay using sandwich ELISA using Alexa Fluor 488 fluorescent dye-labeled detection antibody. (b) Magnetic bead immunoassay of sandwich ELISA using Horseradish peroxidase linked detection antibody.



Scheme 3. Different colorimetry methods for magnetic bead immunoassays. (a) Magnetic bead immunoassay of sandwich ELISA using fluorescent dye-labeled detection antibody. (b) Magnetic bead immunoassay of sandwich ELISA using enzyme-labeled detection antibody.

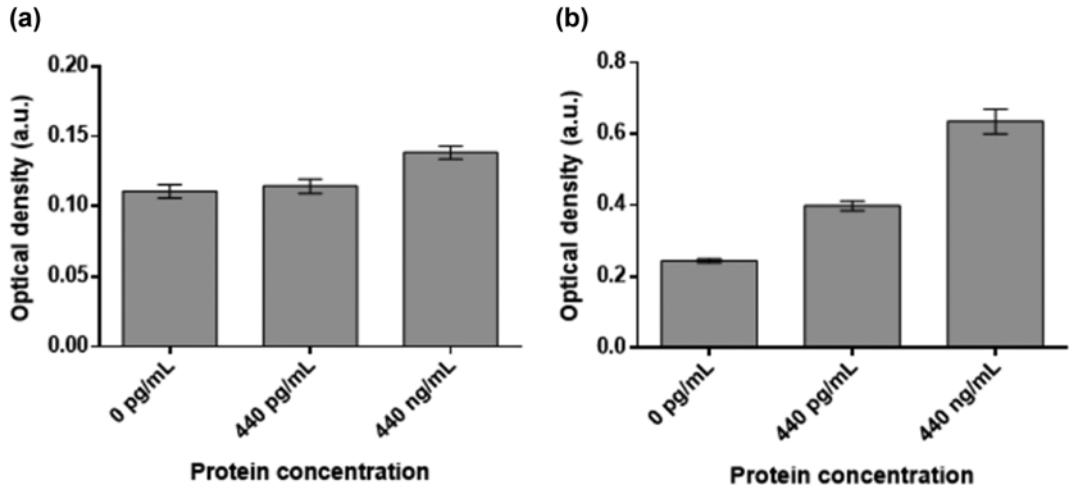


Fig. 4. Comparison of magnetic bead immunoassay sensitivity according to target protein volume. (a) Comparison of absorbance in magnetic bead immunoassay using HRP-linked detection antibody at 150 μL total volume according to SMPD1 protein concentration. (b) Comparison of absorbance in magnetic bead immunoassay using HRP linked detection antibody at 600 μL total volume of SMPD1 protein.

On the other hand, when the magnetic bead based ELISA is performed, since the control and sample groups have very low detection intensities to begin with, there may be no difference in detection intensity between them. This may be caused by an antigen-antibody pairing error, meaning that the detecting antibody cannot bind to the target. Therefore, if a low intensity phenomenon occurs, the first step is to confirm the presence of antigen-antibody pairing. If there is no problem with antigen-antibody pairing, the next troubleshooting strategy is to look at the assay results while increasing the volume of target factor at the same concentration. This is a strategy that can increase the number and probability of binding to the detecting antibody, due to the increase in the total amount of the target factor under the same concentration conditions, thereby increasing the detecting value difference.

Fig. 4(a) and Fig. 4(b) show the results of magnetic bead-based ELISA under the same conditions, comparing the intensity differences of the target factors with the same concentration condition for a lower and higher total volume, respectively. Fig. 4(b) shows higher intensities compared to Fig. 4(a), when the higher volume was reacted with the magnetic bead for the same target factor concentration. As a result, it was possible to achieve better sensitivity. Therefore, when a low intensity phenomenon occurs in the optimization of the concentration-dependent processes for the target factor using magnetic beads, if the sensitivity at the low concentration cannot be determined, a significant detection intensity difference can be obtained for each target concentration by increasing the total volume size of the target molecules.

Note that the use of enzyme-labeled antibodies could be a way to increase the sensitivity of detection more than the use of fluorescently labeled antibodies. However, the system using antibodies labeled with fluorescent dye has the following advantages for the detecting target molecules as well. First, fluorescent dye labeling method is easier to apply than enzyme detecting antibody detecting system for target detection and quantification. For example, fluorescently labeled antibodies emit fluorescence in the spot itself bound to the target, so the application may be extended to fluorescent imaging of the target spot as well as quantification of the target. However, the enzyme-labeled antibody detecting system is a system that measures the absorbance of a product formed by reaction of a substrate with an enzyme labeled on an antibody. It is easy to quantify the target, but it is limited to application to the field of imaging of a target spot.

Also, in the fluorescent dye-labeled antibody detecting system, it is possible to solve errors due to interference phenomenon because there are several kinds of chemical fluorescent dyes with various wavelengths compared to the enzyme-labeled antibody detecting system using substrates. For example, the enzyme-labeled antibody detecting system has a limited number of selectable substrates for the enzyme to be used. Therefore, if other factors with the same absorbance wavelength range as the substrate are present in the detecting system, errors due to the interference phenomenon may occur, making it difficult to accurately quantify target factors. However, in the case of fluorescent dye, a dye having fluorescence of various wavelengths has been developed so that the researcher can select the dye of a specific wavelength band which does not cause the interference by the surrounding factors, thereby enabling sensitive detection of the target factor.

In addition, fluorescent dye-labeled antibody detection system can be used as a biosensor that is required to be detected under various conditions because it can detect more stable in various environments than enzyme-labeled antibody detecting system. Enzyme-labeled antibody detecting system uses enzyme, which is a protein, and there are restrictions on detecting system environment such as temperature and pH which may affect protein denaturation. However, in the case of fluorescent dye, it is basically chemical molecule-based, so it is much less likely to be denatured and more stable than a protein having a three-dimensional structure. Therefore, a fluorescent dye detecting system may be effective for application as a biosensor to detect targets in various environments. In these points, researchers should take careful consider-

ation in choosing detection methods that may be more suitable for their research.

SUMMARY

The problems that may occur when optimizing the magnetic bead immunoassay are as follows. First, there is a phenomenon of over-intensity in which detection intensity is too high at all sample concentrations. Secondly, the detection strength may be too weak for all sample concentrations. In the case of over-intensity, the first strategy is to lower the concentration of the secondary antibody labeled with the marker to reduce the overall intensity and then to determine if there is an intensity difference between the control and the sample group. If there is no difference in intensity between the control and the sample group even though the concentration of the secondary antibody is lowered, the next strategy is to change the blocking method or perform an experiment with a secondary antibody labeled with another signaling probe. In the case of magnetic beads, coating with a solid surface with a biomolecule such as BSA or skimmed milk, techniques widely used in well plate immunoassays may not achieve proper blocking efficiency. In magnetic beads for immunoassays, a functional group capable of linking the antigen or antibody to the bead surface is exposed. This will connect a specific target to the bead surface and chemically quench the remaining functional group. It is possible to obtain a much higher blocking efficiency than conventional biomolecule coating methods, which can reduce the overall over-intensity, thus confirming the intensity of significant differences from the control at low sample concentration conditions. The following strategy can achieve significant intensity differences between the control and sample groups by using secondary antibodies labeled with different labeling factors with different colorimetry methods. At a low sample concentration, even if the labeled antibody binds sensitively specifically to the sample, the intensity emitted from the actual labeled antibody is weak and it may not be confirmed. Therefore, using a different colorimetry method that can amplify intensity emitted from one labeling antibody may overcome the invisibility of sample group intensity due to high background signal. By using this, high intensity in the sample group may be formed, and the intensity difference according to the sample concentration difference can be confirmed sensitively. The resolution of the low intensity phenomenon that can occur during the magnetic bead immunoassay optimization process is to first confirm whether the specific binding between the antibody and the analyte is properly formed. If there is no pairing error between the antibody and the analyte, the subsequent solution is to increase the volume of the analyte in the same concentration condition. Another advantage of magnetic bead immunoassays is that the range of analyte sample volume can be varied, with potential conversion to various assay platforms. The immunoassay method using a conventional well plate has limited volume range for the analytical sample due to the limitation of the solid phase formulation in the well plate. However, the magnetic bead immunoassay can be converted into various assay platforms, which can increase the possibility of enhancing the detection ability of the analyte to be analyzed. By increasing the volume of the analytical sample at the same concentrations, it is possible to establish a higher intensity difference compared to the control, even at a low concentration of the sample, by increasing the relative number and probability of the sample which can react with the detecting antibody. This is one of the effective strategies to overcome the low intensity phenomenon. The development of biosensors using magnetic bead immunoassays has extended the scope of the analysis objects to the detection and quantification of a single protein in the past, to microorganisms that cause various diseases as well as proteins themselves. Considering the expansion of these research fields and the utilization of bead assays in them, further optimization is required to increase the sensitivity and selectivity of analytical samples. Therefore, we anticipate the strategies reported in this work may contribute to the development of better biosensors by more effectively and quickly solving the problems in immunoassay optimization using magnetic beads.

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REFERENCES

1. I. A. Darwish, *Int. J. Biomed. Sci.*, **2**, 217 (2006).
2. G. A. Bonwick and C. J. Smith, *Int. J. Food Sci. Technol.*, **39**, 817 (2004).
3. R. M. Lequin, *Clin. Chem.*, **51**, 2415 (2005).
4. A. H. Wu, *Clin. Chim. Acta*, **369**, 119 (2006).
5. K.-Y. Lien, L.-Y. Hung, T.-B. Huang, Y.-C. Tsai, H.-Y. Lei and G.-B. Lee, *Biosens. Bioelectron.*, **26**, 3900 (2011).
6. Ø. W. Rønning and A. C. Christophersen, *Hybridoma*, **10**, 641 (1991).
7. T. Aytur, J. Foley, M. Anwar, B. Boser, E. Harris and P. R. Beatty, *J. Immunol. Methods*, **314**, 21 (2006).
8. T. Jackson and R. Ekins, *J. Immunol. Methods*, **87**, 13 (1986).
9. P. Nikitin, P. Vetoshko and T. Ksenevich, *Sens. Lett.*, **5**, 296 (2007).
10. S. Gundersen, I. Haagensen, T. Jonassen, K. Figenschau, N. De Jonge and A. Deelder, *J. Immunol. Methods*, **148**, 1 (1992).
11. F. Zhao, Q. Shen, H. Wang, X. Han and Z. Yang, *Food Control*, **79**, 227 (2017).
12. A. Radoi, M. Targa, B. Prieto-Simon and J.-L. Marty, *Talanta*, **77**, 138 (2008).
13. S. Yu, F. Yu, Y. Li, L. Liu, H. Zhang, L. Qu and Y. Wu, *Food Control*, **60**, 500 (2016).
14. D.-H. Jung, K. Min, Y. Jeon, W. Jang and Y. Kwon, *Biochip J.*, **6**, 293 (2012).
15. J. Andreassen, *Immunodiagnostic*, **29**, 22 (2005).
16. R. Perraut, M. Varela, B. Mbengue, M. Guillotte, O. Mercereau-Puijalon and I. Vigan-womas, *J. Immunol. Tech. Infect. Dis.*, **4**, 1 (2015).