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A Novel Protocol for Generating High-Affinity ssDNA Aptamers by Using Alternating Magnetic Fields

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Abstract

Background: Aptamers that have been generated using a SELEX (Systematic Evolution of Ligands by EXponential enrichment) process have been found to have high specificity and affinity with molecular targets, and, as a result, have found use in diagnostic and therapeutic applications. The SELEX protocol generally requires 5 to 15 rounds of selection to generate high-affinity aptamers; however, the standard protocol is labor-intensive and time-consuming. Here, we propose a method for DNA aptamer screening, in which the evolutionary process is completely abandoned.

Methods: The working principle of this new protocol, Magnetic-Assisted Rapid Aptamer Selection (MARAS), is discussed. We used C-reactive protein, a common cardiovascular disease (CVD) indicator, as a molecular target. An externally-applied alternating magnetic field, aided by target-bound magnetic nanoparticles, was used to provide the competitive mechanism for the selection of DNA aptamers with different affinities to the target protein.

Results: The range of the dissociation constants of the screened aptamers was approximately several nMs, depending on the frequency and the field strength of the externally-applied alternating magnetic field. We also compared the diagnostic applicability of the aptamers generated by the proposed MARAS protocol with an enzyme-linked immunosorbent assay (ELISA), using antibodies.

Conclusions: The proposed MARAS protocol efficiently generates aptamers that have high affinity and specificity with molecular targets. In addition, the proposed protocol represents significant time savings, as it can be completed in less than an hour. Furthermore, due to the simplicity of the MARAS protocol, we suggest that the proposed process could be easily automated.

Keywords: Aptamers; SELEX (Systematic Evolution of Ligands by EXponential enrichment); C-reactive protein; magnetic nanoparticle; MARAS; affinity

Introduction

The single-strand SELEX (ss-SELEX) protocol was first described in 1990[1-3]. Since then, many modifications have been proposed, but the basic principles of the SELEX process have remained largely unaltered. The SELEX protocol involves multiple rounds of a process that repeats incubation, separation, elution, amplification and purification steps in order to search for nucleotides that are better able to bind to a desired target molecule. First, a randomized DNA library is mixed with target molecules. Second, DNA that is bound to the target molecule is separated from unbound DNA. Third, the bound DNA is amplified by PCR to produce an aptamer-enriched DNA library. Lastly, single strand oligonucleotides are purified from the PCR product. The whole procedure is repeated for several rounds, starting with the enriched library obtained in the preceding round. Typically, between 5 to 15 SELEX selection cycles must be performed until no further enrichment of the functional nucleic acid species is detectable. Generally, multiple rounds of selection to isolate aptamers with sufficient specificity and binding affinity (e.g., nanomolar dissociation constant, Kd) to the desired target molecules are needed. SELEX technology therefore requires a significant investment of resources, time, and analytical equipment, software and systems. As a result, the selection protocol has subsequently been reduced to an automated *in vitro* process[4,5]. Also, several interesting and functional strategies have been developed to facilitate this lengthy procedure, including capillary electrophoresis (CE-SELEX)[6, 7], microfluidics SELEX (M-SELEX)[8] and one-step MonoLEX[9]. The CE-SELEX procedure uses the high efficiency of capillary electrophoresis to separate aptamers bound with targets, which results in a significant shift in electrophoretic mobility. The aptamers that are obtained after a few (i.e., 1 to 4) CE-SELEX selection rounds have a high affinity to target molecules with Kd at the nanomolar level. However, the CE-SELEX protocol is less effective in screening aptamers that bind with small molecules, because small molecules do not induce sufficient shift for separation. The M-SELEX protocol consists of a continuous-flow, magnetically-activated, chip-based separation (CMACS) device and a magnetic, nanoparticle-assisted SELEX. In the M-SELEX protocol, a localized magnetic gradient field via ferromagnetic patterns is integrated into the microchannel to separate aptamers bound to target-coated, magnetic beads from unbound oligonucleotides. The Kd of aptamers that are isolated via a single round of M-SELEX is 33±8 nM. However, M-SELEX requires precise microfludic technology and numerous instruments that are not common in biochemistry and medical laboratories. The one-step MonoLEX approach uses affinity chromatography, which is carried out on resin-bound, target molecules with an extensive washing procedure to elute non-binding and low-binding oligonucleotides, and physical

segmentation of the affinity column. Under adequate chromatographic conditions, such as low laminarity, sufficient capacity and homogeneity of the resin, high affinity binding aptamers are bound to the target. A physical partitioning of the affinity resin is required to verify for different targets, to ensure the binding affinity of the screened aptamers. Therefore, it is difficult to control the outcome of the MonoLEX process. Other modified SELEX procedures that are less commonly used include Photo-SELEX[10], SPIEGELMER-Technology[11], and Genomic SELEX [12].

The advantages of aptamers have drawn the attention of scientists, but the tedious and time-consuming nature of the traditional SELEX process has imposed limits on the practical uses of the technology. Although modifications exist that reduce some of the time involved in the SELEX process by decreasing the number of evolution cycles needed, none of the existing protocols are simple enough to enable end users to rapidly screen for suitable aptamers for desired applications. Moreover, due to the multiple repeated cycles required for the process, automation and miniaturization are very difficult. Thus, a new protocol which abandons the evolutionary process and is able to efficiently and rapidly generate aptamers with high affinity and specificity is urgently needed.

Here, we propose a novel protocol for screening suitable aptamers to target molecules from a randomized oligonucleotide library. The working principle of the protocol is discussed and the effectiveness and feasibility of the protocol is demonstrated.

Working principle of MARAS

Generally, aptamer-generating procedures involve 3 steps: (1) material preparation, (2) screening, and (3) post-analysis to characterize selected aptamers, which includes cloning, PCR amplification and dissociation constant calculation. While the first and last steps for both the SELEX and proposed MARAS procedures are the same, in the second step (screening), the proposed method eliminates the repeated selection rounds required by the SELEX procedure. In this study, we demonstrate the viability of such a streamlined, MARAS procedure. The MARAS procedure is illustrated by the flowchart depicted in Figure 1a. As shown in Figure 1a, the screening procedure of the proposed MARAS protocol is streamlined to involve just 2 steps: (1) the application of a proper alternating magnetic field to the sample, and (2) the execution of a negative selection.

The working principle of the proposed protocol is based on providing a competitive mechanism to differentiate oligonucleotide sequences of varying binding affinities to target molecules. By varying the strength of the competitive mechanism, it was hypothesized that the different binding affinities of oligonucleotide sequences

to the target molecules could be differentiated. We theorized that an externally-applied alternating magnetic field, coupled with the aid of target-bound magnetic nanoparticles, would be able to provide a competitive mechanism for the selection of DNA aptamers with different affinities to the target molecules.

It was thought that when mixing randomized oligonucleotide sequences with a reagent containing target-coated, bio-functionalized magnetic nanoparticles, a portion of the oligonucleotide sequences would bind with the target molecules to form aptamer-target-magnetic nanoparticle bound mixtures. The bound mixtures inside the reagent would be disturbed by an externally-applied, oscillating magnetic field through the magnetic torque generated by the interaction between the dipole moment of the magnetic nanoparticles and the magnetic field. If the magnetic field was generated by a solenoid with an alternating current, then the field direction inside the solenoid would always be along the axis of the solenoid with an alternating magnitude. Then, it was theorized that if the bound mixtures were placed inside the solenoid, due to the interaction, the dipole moment of the magnetic nanoparticles would tend to align with the field direction. On the other hand, the thermal agitation would tend to misalign the dipole moment of the magnetic nanoparticles with the field direction. We hypothesized that as the magnetic field amplitude alternated from positive maximum to zero and then to negative maximum, the magnetic torque and the thermal agitation would cause the magnetic nanoparticles to oscillate around the axis of the field direction. As the magnetic nanoparticles oscillated in a viscous fluid, a dissipative torque opposite to the direction of the magnetic nanoparticles' motion would be generated. Thus, the magnetic torque would act as a driving torque and the dissipative torque would act as an opposite torque. As a result, a stretch force would generate and act on the bond among the oligonucleotide sequences and the target molecules, which would have been firmly bound to the magnetic nanoparticles through the strong binding of the conjugation pair, streptavidin of bio-functionalized magnetic particles and biotin of biotinylated target molecules. It was hypothesized that this stretch force would provide a competitive mechanism and thus, by enhancing the competitive mechanism, aptamers with higher affinity would be obtained. In other words, the aptamers with low binding affinity for the target molecules would detach from the target-bound magnetic nanoparticles, if the competitive mechanism overcame the binding strength between the aptamers and the targets. It was also thought that after being detached from the target-bound magnetic nanoparticles, the aptamers with low binding affinity toward the target molecules could be removed with a magnetic stand.

Figure 1b illustrates in detail the steps in a tube-based process, of which the library is assumed to contain non-binding (colored gray and green), low-binding (colored red) and high-binding (colored blue) oligonucleotides. Briefly, the

non-binding oligonucleotides can be removed by magnetic separation after the library has been incubated with target-coated biofunctionalized magnetic particles. Next, the low-binding oligonucleotides can be detached from these particles by applying an alternative magnetic field of a proper frequency and strength. Finally, the detached low-binding oligonucleotides can be removed by magnetic separation.

Experimental setup for MARAS

The experimental setup used to demonstrate the feasibility of the MARAS process is described below. The experimental setup consisted of (1) a magnetic field; (2) a power amplifier (LPA 05, Newtons4th Ltd, Mountsorrel, Charnwood, UK); (3) a signal generator (WF1944B, NF Corp, Yokohama, Japan); and (4) a LABVIEW computer program. An externally-applied alternating magnetic field was used in the MARAS procedure, which was generated by a single excitation solenoid driven by a current generator unit, schematically shown in Figure 2. The sample was placed inside the solenoid. The LABVIEW program was used to send an alternating signal, into a power amplifier, via a NI BNC-2110 capture box. The field strength was calibrated by using a gauss meter. Due to the application of the alternating magnetic fields during the screening process, we refer to this protocol as AC-MARAS in this paper. It must be noted that during the application of an AC magnetic field, heat will be generated by the solenoid. A water bath can be used to disperse heat to prevent heat damage to the power supply, solenoid and the test sample. In each experiment discussed in this study demonstrating the MARAS process, the solenoid and test tube containing the sample solution were partially immersed in a water bath and the temperature of the water bath was kept at room temperature during the course of the entire process.

Field-dependence of the MARAS procedure

One nanomole of the 20N randomized oligonucleotide library (Electronic Supplementary Information: ESI) was diluted to 10 μL with BD buffer (ESI) in a micro-tube, heated to 95°C for 5 minutes and then snap cooled at 4°C. The CRP-MNPs (ESI), obtained by magnetic separation from 5 μL of CRP reagent (ESI), were added into the micro-tube and incubated for 30 minutes at room temperature. The unbound nucleotides were removed with a magnetic stand, and the bound mixture was washed 3 times with 1 mL of BD buffer. 100 μL of BD buffer was added to re-disperse the bound mixture in the micro-tube, and the micro-tube was placed in the setup depicted in Figure 2. The applied magnetic field was an alternating magnetic field and the field strength was calibrated by a gauss meter at the sample location.

Before demonstrating the proposed MARAS protocol, the effect of the field parameters of the externally-applied alternating magnetic field on the affinity of selected aptamers to the target molecules was first evaluated in order to obtain a

preferable field condition for the later demonstration of the proposed protocol. Specifically, the effect of the field frequency and the field strength on aptamer affinity, which is expressed by the dissociation constants to the target molecules, was examined.

For a frequency-dependent study of the AC-MARAS protocol, the re-dispersed bound mixtures were subjected to a constant magnetic field strength (25 gauss) and a series of field frequencies (e.g., 2, 20, 200, 2 K, 20 K, and 200 KHz) through the setup shown in Figure 2. The bound mixture was initially subjected to an alternating magnetic field of 2 Hz for 10 minutes at room temperature and was stirred every 2.5 minutes by pipetting, in order to avoid agglomeration due to the action of the magnetic field on the magnetic nanoparticles. A magnetic separation was performed to collect supernatant. Consequently, 100 μL of BD buffer was added to re-disperse the retained bound mixture, and the mixture was subsequently subjected to a magnetic field with the next frequency in the series. The above procedure was repeated until a magnetic field with the final frequency (200 KHz) was applied. After the process was completed with the applied magnetic field with the final frequency, magnetic separation was used to separate the supernatant, and the bound mixture was retained. Finally, 100 μL of BD buffer was added to re-disperse the retained bound mixture and heated to 95°C for 5 minutes to elute the aptamers from the CRP-MNPs. Magnetic separation was performed to collect the supernatant. Then, the collected supernatant was subjected to a negative selection, which is described below. After a negative selection, the final supernatant was named as >200K. In this paper, we refer to the above-mentioned selection, which involves the application of an alternating magnetic field, as a positive selection. Since the target used was CRP-streptavidin, magnetic nanoparticles and the aptamers could be selected from those bound to SA-MNPs (ESI) instead of the CRP (ESI). As a result, all the collected supernatants were subjected to a negative selection, which was performed by using SA-MNPs to eliminate any possibility of aptamers that could bind to the SA-MNPs. For a negative selection, SA-MNPs, obtained by magnetic separation from 5 μL of SA reagent (ESI), were added to each supernatant and incubated for 30 minutes at room temperature. Magnetic separation was performed to remove the aptamer-streptavidin-magnetic nanoparticles, and the supernatant was collected. The supernatants, which were obtained from each experimental step, with various field frequencies after a negative selection, were named for the corresponding frequency. All the collected supernatants were precipitated with 1 mL of 100% cold alcohol for further analysis, such as PCR amplification, cloning and sequencing.

For a field strength dependent investigation, an AC magnetic field at a fixed frequency (100 KHz) with a successive field strength (e.g., 12.5, 25, 50, 100, 200 and

400 gauss) was performed via the setup of Figure 2 (HAC-MARAS). The experimental procedure was similar to that for the frequency-dependent analysis. In addition, a similar naming scheme for the collected supernatants was adopted. The corresponding supernatants after a negative selection were collected and precipitated with 1 mL of 100% cold alcohol for further PCR amplification, cloning and sequencing.

Demonstration of the AC-MARAS procedure

In order to further demonstrate the ease and the viability of the AC-MARAS protocol for the selection of aptamers that have high binding affinity to target molecules, a one-shot MARAS (OS-MARAS) was performed using the setup shown in Figure 2. The bound mixture of a 20N library with CRP-MNPs was prepared according to the procedure specified earlier in this paper. The re-dispersed bound mixture was subjected to an alternating magnetic field of 400 gauss and 200 KHz for 10 minutes at room temperature and stirred every 2.5 minutes by pipetting. After a magnetic separation, the supernatant was removed and the retained bound mixture was re-dispersed with 100 μL of BD buffer. Afterward, the mixture was heated to 95°C for 5 minutes, and a magnetic separation was performed to collect the supernatant. Then, a negative selection was performed to eliminate the possible SA-MNP bound aptamers. Another magnetic separation was performed to collect the supernatant, which was then precipitated with 1 mL of 100% cold alcohol for further PCR amplification and cloning. Two aptamers were picked for the following sequencing, secondary structure prediction, and binding affinity calculations.

RESULTS AND DISCUSSION

Characterization of aptamers from the MARAS procedure

A library of 20N was used for AC-MARAS selection. The preparation of the bound mixture and the setup of alternating magnetic field were as described above. The magnetic field strength used was 25 gauss, via the setup of Figure 2. Two of the aptamers screened from each step of the MARAS selection corresponding with the alternating magnetic field frequencies were named as 20N AC F2(-2, -4), 20N AC F20(-2, -4), 20N AC F200(-1, -2), 20N AC F2K(-1, -4), 20N AC F20K(-1, -3), 20N AC F200K(-1, -2), and 20N AC>200K(-1, -2). The results of the sequences of the selected 20N aptamers are listed in Supplemental Table 1 (Table. S1). The representative sequencing figures, predicted secondary structures (ESI Figure S1 a) were predicted by using Mfold (ESI Reference). The result illustrated that the possible conserved motifs that might interact with CRP protein was a central circle connected with 2-3 stem loops. The dissociation constants (Kds) were calculated by q-PCR and a non-linear fitting curve (ESI Reference). The lowest Kd value of the 20N aptamer,

screened by AC-MARAS at a constant field strength of 25 gauss with a series frequency, was 8.35 nM for the 20N AC>200K-2 aptamer. Figure 3(a) shows that the dissociation constants (Kds) of the selected 20N aptamers decreased according to the frequency of the applied alternating magnetic field for AC-MARAS. Accordingly, the high binding affinity of the aptamer could be screened by increasing the frequency of MARAS. This demonstrated that the affinity to the target molecule of the screened aptamers by MARAS was dependent on the frequency of the applied alternating magnetic field.

Consequently, a magnetic field strength dependent analysis was performed with a field frequency at 100 KHz and a series field strength (e.g., 12.5, 25, 50, 100, 200 and 400 gauss) in the AC-MARAS. The experimental processes were as described above. The corresponding aptamers that were screened by the field strength dependent AC-MARAS were named as, 20N HAC 12.5g(-1, -2), 20N HAC 25g(-1, -2), 20N HAC 50g(-2, -9), 20N HAC 100g(-1, -2), 20N HAC 200g(-2, -3), 20N HAC 400g(-1, -5) and 20N HAC >400g(-1, -4). The sequence results of the aptamers are listed in Supplemental Table 2 (Table S2). The sequence results, predicted secondary structures (ESI Figure S1 b) and calculated dissociation constants of 20N aptamers were determined by using the method described above. As showing in Supplemental Figure 1 b (Figure S1 b), the homologous information of obtained sequences was similar to those described previously (ESI Figure S1 a). The lowest Kd value of a 20N aptamer that was screened by a corresponding alternating field strength (e.g., 12.5, 25, 50, 100, 200, and 400 gauss) at a constant field frequency of 100 KHz was 5.67 nM for the 20N HAC 400g-1 aptamer. Figure 3(b) demonstrates that the dissociation constants (Kds) of the selected aptamers decreased according to the applied magnetic field strength of HAC-MARAS.

Accordingly, it was found that an aptamer bound to a target molecule with a desired range for the dissociation constant can be selected by properly choosing a lower cutoff and an upper cutoff for the frequency and strength of the applied alternating magnetic fields via the MARAS protocol.

Demonstration of MARAS platform

Based upon the results from the field dependent analysis, the preferable conditions for the one-shot MARAS was shown to be 400 gauss and 200 KHz, via the setup shown in Figure 2. The material preparation and the experimental procedure were as described above. The selected 20N aptamers that were screened by one-shot MARAS were named OS-AC-20N-1 and OS-AC-20N-3, and the sequence results are shown in Supplemental Table 3 (Table S3). For the one-shot MARAS, the sequence results, predicted secondary structures, and calculated binding affinities of the selected

aptamers were determined by the methods described before (data no show). The Kds of the aptamers from the one-shot MARAS experiments were 5.96 nM for OS-AC-20N-1 and 5.70 nM for OS-AC-20N-3. According to the results, the average dissociation constant of the selected aptamers was 5.83±0.18 (5.96, 5.70) nM. These results indicate that the values of the Kds of the aptamers screened by the one-shot MARAS could easily reach a single digital nanomolar concentration and are consistent with those of our previous results (Figures 3).

Direct observation of affinity selectivity and binding specificity to the target of aptamers selected by MARAS

To demonstrate the affinity selectivity and binding specificity of the screened aptamers by using the MARAS protocol, an alternating magnetic field with a strength of 25 gauss and a frequency of 200 KHz was used. Two aptamers, 20N AC F200K-1 and 20N AC >200K-2, which were isolated previously with the AC-MARAS protocol, were used. The dissociation constants of the aptamers were 12.49 and 8.35 nM, respectively. The preparation of the FITC-labeled aptamer was as described (ESI). The FITC-labeled aptamer was incubated with CRP reagent and SA reagent, separately. The re-dispersed bound mixtures of the FITC-labeled aptamers with CRP reagent and SA reagent were observed with a fluorescence microscopy, and the corresponding images are shown in Figure 4. No fluorescence was observed when SA reagent was used as shown in Figures 4a and 4d. However, fluorescence was observed when CRP reagent was used as a target, as shown in Figures 4b and 4e. These results demonstrate that a selected aptamer can specifically bind to CRP protein and cannot bind to SA-MNPs. The bound mixtures of the FITC-labeled aptamers with the CRP reagent were subjected to an alternating magnetic field (25 gauss/200 KHz). After being washed, detached aptamers were removed by using the magnetic stand. The bound mixture of the FITC-labeled aptamer with CRP reagent was re-dispersed and observed again by using a fluorescence microscopy. Our results showed that no fluorescence was observed in the case of the 20N AC F200K-1 aptamer after the alternating magnetic field was applied (Fig. 4c). On the other hand, the fluorescence was still observed in the case of the 20N AC >200K-2 aptamer (Fig. 4f). Furthermore, the bound mixtures of the aptamers (FITC-labeled 20N HAC F400g-5 and 20N HAC>400g-4), which were isolated with the HAC-MARAS protocol described previously, with the CRP reagent and SA reagent being prepared similarly to that as in the procedure as described above. The bound mixtures were subjected to an alternating magnetic field (400 gauss/100 KHz). The results are shown in Figure 5. As expected, no fluorescence was observed in Figures 5a and 5d, but fluorescence were observed for the FITC-labeled aptamers bound with CRP-MNPs as shown in Figures

5b and 5e. No fluorescence was observed for the bound mixture for 20N HAC F400g-5 aptamers (Fig. 5c), but fluorescence was observed for 20N HAC >400g-4 aptamers (Fig. 5f). These results demonstrate that, by altering either the frequency or strength of the externally-applied alternation magnetic field in the MARAS process, aptamers with different binding affinities to the target molecules can be differentiated. It is worthy to note that before taking fluorescence images, the green spots in these two figures (Figures 4 and 5) were pulled by using a magnet to assure these green spots containing the target-bound magnetic nanoparticles binding with the FITC-labeled aptamers.

Comparison of assaying target concentration based on aptamers and antibodies by using enzyme-linked immunosorbent assay

An aptamer-based ELISA was performed as described previously to verify the applicability of the aptamers by assaying the CRP concentration, and the result was compared with that of using an antibody-based ELISA. Two aptamers selected from the one-shot MARAS process, OS-AC-20N-1 and OS-AC-20N-3, were used. The dissociation constants of the aptamers were 5.96 and 5.70 nM, respectively. In the assay, CRP was immobilized on an ELISA microplate (ESI), and then an individual biotin-labeled aptamer (ESI) was added with a fixed, overdosed concentration to ensure a saturated value for the binding site on the well. The result is shown in Figure 6. Figure 6 shows the binding between the selected aptamers (OS-AC-20N-1 and OS-AC-20N-3) and CRP as visualized by the microplate reader. In Figure 6, the optical intensity from assaying different concentrations of the CRP by using an aptamer-based ELISA was compared with the results from when an antibody-based ELISA was used. Figure 6 clearly illustrates that the ELISA result by using an aptamer-based detection kit is similar to that of the result from using an antibody-based detection kit in assaying the CRP concentration.

Conclusion

According to our experimental results, aptamers isolated through the MARAS protocol can interact with target molecules. Also, the dissociation constants selected through the MARAS protocol are magnetic field-dependent. Specifically, it was found that the value of a dissociation constant decreases as the field frequency and the field strength increase. In addition, it was shown that the dissociation constants of single strand aptamers binding with targets could easily reach a single digital nanomolar concentration by applying an alternating magnetic field with a proper frequency and strength for the MARAS protocol. In addition, an aptamer bound to a target molecule with a desired range for the dissociation constant can be selected by properly choosing lower and upper cutoffs for the frequency and strength of the applied

magnetic fields via the MARAS protocol. As compared to the traditional ss-SELEX process, the MARAS protocol described in this study consumes fewer supplies and can be completed in less than an hour, as compared to the several weeks to several months necessary to complete the traditional ss-SELEX protocol.

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Figure legends

Figure 1. (a) The flowchart of AC-MARAS. (b) The detail steps of MARAS procedure.

Figure 2. Schematic diagram of the AC-MARAS apparatus.

Figure 3. The Kd results of 20N aptamers screened by MARAS. (a) Field frequency dependence, (b) Field strength dependence.

Figure 4. Representative images of fluorescence with the binding affinity of selected aptamer with CRP reagent. (a). FITC-20N AC F200K-1 aptamer blinding with SA-MNP. (b). FITC-20N AC F200K-1 aptamer binding with CRP-MNP. (c). FITC-20N AC F200K-1 aptamer binding with CRP-MNP after having been subjected to an AC magnetic field (25 Gauss/200 KHz). (d). FITC-20N AC >200K-2 aptamer binding with SA-MNP. (e). FITC-20N AC >200K-2 aptamer binding with CRP-MNP. (f). FITC-20N AC >200K-2 aptamer binding with CRP-MNP after having been subjected to an AC magnetic field (25 Gauss/200 KHz).

Figure 5. Representative images of fluorescence with the binding affinity of selected aptamer with CRP reagent. (a). FITC-20N HAC 400g-5 aptamer blinding with SA-MNP. (b). FITC-20N HAC 400g-5 aptamer binding with CRP-MNP. (c). FITC-20N HAC 400g-5 aptamer binding with CRP-MNP after having been subjected to an AC magnetic field (400 Gauss/100 KHz). (d). FITC-20N HAC >400g-1 aptamer binding with SA-MNP. (e). FITC-20N HAC >400g-1 aptamer binding with CRP-MNP. (f). FITC-20N HAC >400g-1 aptamer binding with CRP-MNP after having been subjected to an AC magnetic field (400 Gauss/100 KHz).

Figure 6. Comparison of the results in assaying CRP concentration using aptamer-based and antibody-based ELISA.