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New Aptamer Pairs as a Candidate for the Rapid Detection of Food Pathogen, *Salmonella enteritidis*

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Food pathogen

Abstract: Food pathogens are crucial for human and animal health. *S. enteritidis*, enteric bacteria, is one of the most popular pathogen and causes salmonellosis. Vomitting, diarrhea, fever and abdominal cramps are typcial symptoms occuring at 12-72 hour after the infection and can cause to death. Food based infections in the USA and EU are primarily caused by *S. enteritidis*. Thus, early and rapid detection of bacteria is always highly significant. In this sense, lateral flow assays (LFAs) are notably advantageous with regards to the obtaining rapid results and practical use. LFAs are commonly prepared by antibodies and aptamers. Aptamers are single stranded DNA and RNA molecules and bind to the target molecules, selectively. Gold nanoparticles (AuNPs) are the most common labels used for LFAs. In this work, it is aimed to develop the aptamer paired strip assay (APSA) platform by using aptamer pairs specific to *S. enteritidis*. It was observed that 1st aptamer pairs are suitable for developing the LFAs for the recognition of *S. enteritidis* compared to the other pairs and M180 membrane was found as suitable membrane in terms of the flow rate and positive results.

Gıda Patojeni *Salmonella enteritidis*'in Hızlı Teşhisi için Aday Yeni Aptamer Çiftleri

Anahtar Kelimeler
S. enteritidis,
Aptamer pairs,
Lateral flow assay,
Food pathogen

Öz: Gıda patojenleri insan ve hayvan sağlığı için çok önemlidir. Enterik bir bakteri olan *S. enteritidis* en önemli patojenlerden biridir ve salmonellosise sebep olur. Kusma, ishal, ateş ve abdominal kramplar enfeksiyondan 12-72 saat sonra oluşan tipik semptomlardır ve ölüme sebep olabilir. ABD ve Avrupa'da gıda bazlı enfeksiyonlar öncelikle *S. enteritidis*'ten kaynaklanır. Bu nedenle bakterinin erken ve hızlı teşhisi her zaman oldukça önemlidir. Bu anlamda yanal akışlı testler (LFAs) hızlı sonuçlar alma ve pratik kullanım açısından oldukça avantajlıdır. LFA'ler yaygın olarak antikor ve aptamerler ile hazırlanır. Aptamerler tek zincirli DNA ve RNA molekülleridir ve hedef moleküle seçici şekilde bağlanır. Altın nanoparçacıklar (AuNPs) LFA'lerde en yaygın kullanılan işaretleyicilerdir. Bu çalışmada *S. enteritidis*'e özgün aptamer çiftleri kullanılarak aptamer çiftli strip test (APSA) platformu geliştirilmesi amaçlanmıştır. 1. aptamer çiftinin diğer çiftlerle karşılaştırıldığında *S. enteritidis* teşhisi için LFA geliştirilmesinde uygun olduğu gözlenmiştir ve M180 membran akış oranı ve pozitif sonuçlar açısından uygun membran olarak bulunmuştur.

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1. Introduction

Salmonella spp. having about 2300 serotypes is one of the most important bacteria which causes to enteric diseases for human and animals. Gastroenteritis, enteric fever and sepsis are common disorders caused by

Salmonella. Most of the infections resulted by Salmonella are arised by food and transmission to human is generally occurred with meat, chicken, egg, poultry and pig. So, it can cause to salmonellosis via invading the digestive system of host with contaminated nutrition and might be resulted with death. Besides, Salmonella infections may be resulted with economic loss at food industry, critically [1]. Almost half of the infections of digestive system seen in the USA in every year are caused by contaminated food by Salmonella. According to the annual report for zoonoses of European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), nearly one in three foodborne outbreaks in the EU in 2018 were caused by Salmonella and Salmonellosis was the second most commonly reported gastrointestinal infection in humans in the EU (91 857 cases reported) after campylobacteriosis (246 571). Among the 5146 food and waterborne outbreaks, Salmonella was the most commonly detected agent and *S. enteritidis* caused one in five outbreaks [2] which is similar with the report in 2016 [3]. Additionally, 15 EU/EEA countries reported 656 confirmed cases and 202 probable cases for *S. enteritidis* from 2017 to 2020 [4]. Thus, since *S. enteritidis* is one of the most common species causing to food poisoning, its rapid detection is crucial. However, conventional methods including culturing in selective medium, serological and biochemical assays take 4 days and need trained personnel [5]. The delay in detection may also increase the infection risk. Although polymerase chain reaction (PCR) [6], immunomagnetic [7] and biosensor [8] based detections were reported, the need of more reliable extraction methods, further experimental steps and advanced devices, and sample loss are some limitations. Thus, rapid and sensitive detection is always become attractive. In that case, LFAs which are immunochromatographic assays composed of four membranes and ready to use point of care assays have some advantages compared to the those methods. They are simple diagnostic dipstick assays developed by various reagents including antibodies, aptamers and nanoparticles [9][10] as a commercial since 1980. Long shelf life, practical and easy to usage, specificity and high sensitivity, integration with electronical and reading systems, rapid manufacturing and market presence are some advantages of LFAs. AuNPs are most widely used labels for LFAs. Because it allows to naked eye analysis and easy surface modification for immuno-analysis and biosensor applications [11][12].

Aptamers are single chain DNA or RNA molecules binding to their targets with high affinity such as proteins as a defined architectures and having long shelf life [13][14]. They are discovered by systematic evolution of ligands by exponential enrichment (SELEX) and alternately partitioned for target binding. Since they are oligonucleotides aptamers can be assembled into supramolecular multi-component structures using hybridization. The adaptation of aptamers to biosensor and novel diagnostic systems become popular in 2000s. Although the usage of aptamers in LFAs is increasing in recent years [15], researches are still limited because of the technical and application procedures. Using aptamers in LFA is generally based on aptamer pairs while various reagents can be used along with the aptamers, and the aptamer based LFAs have some advantages compared to the antibody based LFAs. Because two antibodies may not be paired succesfully [16], labelling with antibodies needs to sensitive pH and salt conditions, and has storage problems restricting the process. Since the endurance of antibody is a crucial problem [17] alternative recognition pairs should be replaced for sandwich assays. Thus, a few aptamer based LFAs developed for the recognition of *S. enteritidis* were reported in the literature. However, capture aptamers used for the assays are different from each other, further experimental steps such as isothermal strand displacement amplification or advanced instruments are needed for the analysis and affinity of aptamers to the target may be low [18][19][20]. Therefore, new aptamer pairs as a candidate for the rapid and sensitive detection of *S. enteritidis* are needed for developing point of care tests and various detection systems.

In this work, two aptamers specific to *S. enteritidis* which were recently reported in the literature with high affinity were used for developing the LFAs by preparing 4 aptamer pairs in order to see the potential usage of these aptamers for point of care tests..

2. Material and Method

2.1. Chemicals

$HAuCl_4 \bullet xH_2O$ (Fluka), nitrocellulose membranes (M240, M180, M120), sample pad and absorbant pad, glass fiber conjugate pad (Millipore), Tryptic Soy Broth (Sigma) and streptavidin (Sigma) were used. Sodium tricitrate was from Applichem. Two aptamers, Crn-2 and Crn-1, specific to *S. enteritidis* [13] were used as thiol or biotin modified and the primer regions were labelled as bold. Thiol modified aptamers which are Crn-2 SHA: 5'Thio/ **AAGGGCTGGCTGGGATGGA** TGT AAG AAG GGA GGA AAG GAC CTA AGA CCT GCT ATA TTG CGA **TCACTCCACGGACCCCACT**-3'and Crn-1 SH: 5' Thio/ **AAGGGCTGGCTGGGATGGA** CCC TCC CGA AAC GAG CTG TCT CTT AAC GGA AGC TAA TCT GCC **TCACTCCACGGACCCCACT**-3' were called as capture aptamers. Biotin modified aptamers which are Crn-1-Bio: 5' Bio-**AAGGGCTGGCTGGGATGGA** CCC TCC CGA AAC GAG CTG TCT CTT AAC GGA AGC TAA TCT **GCCTCACTCCACGGACCCCACT**-3' and Crn-2 Bio: 5' Bio **AAG GGC TGG CTG GGA TGG A** TGT AAG AAG GGA GGA AAG GAC CTA AGA CCT GCT ATA TTG CGA **TCA CTC CAC GGA CCC CAC T**-3' were called as detector aptamers, and biotin modified sequences complementary to two capture aptamers were purchased from

Integrated DNA Technologies. *S. enteritidis* and *E.coli* were from NanoBiz R&D (Turkey). Nanodrop 2000 UV–Vis spectrophotometer (Thermo Scientific; Waltham, MA, USA) and multiscan plate reader (Biotek-Epoch/2 microplate reader) were used for obtaining the spectra from AuNPs and conjugates. Malvern Zeta Potential instrument was used for getting the size and zeta potential of both the naked and conjugated AuNPs.

2.2. Methods

2.1. Gold nanoparticle synthesis

AuNPs were synthesized according to the sodium citrate reduction and concentrated. Briefly, 0.01% $HAuCl_4.xH_2O$ was boiled in sterile conical flask and 1% sodium citrate was added into the solution [21]. After changing the color of solution from black to reddish in a 2 min, it was allowed to further boiling for about 10 min to complete the reduction before filter sterilization. UV–Vis spectra was measured for determining the max absorbance of surface plasmons (λ_{max}) of AuNPs. In order to make a concentrated AuNPs, colloidal gold solution was centrifuged for 30 minutes. Then, 5 times concentrated AuNPs, 5X AuNPs, were stored at 4˚C for further use.

2.2. Gold nanoparticles and capture aptamer conjugations

After preparation of the stock solutions of two aptamers (Crn-2 and Crn-1), 0.2µM, 2µM and 4µM of the capture probes were used for the conjugation and compared for the assay efficiency. To activate the disulphide bonds, aptamers were treated with Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) at room temperature, firstly. Then, this solution was added into 1 ml of AuNPs solution and incubated at room temperature for the conjugation. Ultrapure water was used as a negative control. After the incubation, phosphate buffer saline (PBS) was added into the solution for the stability of AuNPs and incubated for overnight. Then, solution was centrifuged at 12000 rpm and pellet was resuspended in 20 mM sodium phosphate buffer containing BSA, %0,25 Tween 20 and sucrose. Then, solution was re-centrifuged and washed with the same buffer as twice and prepared conjugate was stored at 4˚C after resuspending in the same buffer. To develop the sandwich assay two conjugates, conjugate 1 and conjugate 2, and four aptamer pairs using these conjugates were prepared, separately and shown in Table 1.

Table 1. AuNPs-Aptamer conjugates and aptamer pairs used for the sandwich assay on LFAs

Conjugate 1 (Crn-2 SHA/AuNPs)	Crn-2 SHA: 5'Thio/ **AAGGGCTGGCTGGGATGGA**TGT AAG AAG GGA GGA AAG GAC CTA AGA CCT GCT ATA TTG CGA **TCACTCCACGGACCCCACT-3'**
Conjugate 2 (Crn-1SH/AuNPs)	Crn-1SH: 5'Thio/ **AAGGGCTGG CTGGGATGGA**CCC TCC CGA AAC GAG CTG TCT CTT AAC GGA AGC TAA TCT GCC **TCACTCCACGGACCCCACT-3'**
1st aptamer pairs	Conjugate 1/Crn-1 Bio
2nd aptamer pairs	Conjugate 2/Crn-2 Bio
3rd aptamer pairs	Conjugate 1/Crn-2 Bio
4th aptamer pairs	Conjugate 2/Crn-1 Bio

2.3. Preparation of the target bacteria

The concentration of *S. enteritidis* was determined by plate counting method as 10^{10} cfu/ml. Then bacteria was cultured in Tryptic Soy Broth (TSB) and grown at 37˚C for 16 hour at 150 rpm. They were collected at early stationary phase and centrifuged at 5000 rpm for 5 minutes. After washing with PBS, they were suspended in running buffer (RB) and used for loading to the strips. Four different RBs were experienced in this work and called as RB 14, RB 1, RB 13 and RB. RB 14 is 20 mM Tris, 50 mM NaCl, 5 mM KCl, 5 mM MgCl2, 2 mM CaCl2, 0.1 mM BSA, 1.7% Triton X100 [22]; RB 1 is 10X SSC/1% BSA; RB 13 is 10 mM Tris, 120 mM NaCl, 20 mM CaCl2, 5 mM KCl, 0.1% Tween 20, 1% PEG 6000, 2% sucrose, 5% methanol [23] and RB (Binding buffer) is PBS containing 4.5g/L glucose, 5mM MgCl2, 1 mg/ml BSA [13].

2.4. Preparation of the lateral flow assay

The width of the strip tests was adjusted to 0.4 cm and the strip components were manually placed as specified earlier [24]. All the solutions for membrane moisturizing were filter sterilized before usage. Three types of solutions were separately prepared for sample pad soaking to make a comparison. They are 1) 0.05M Tris-HCl, 0.25% TritonX100, 0.15M NaCl [25], 2) 0.5% TritonX 100, 1% BSA, 2% sucrose, 50mM boric acid [18] and 3) 0.01M PBS, 3% BSA, 0.05% Tween 20 [26]. Sample pad was dried at 37˚C and placed on the strip. To prepare a conjugate pad two principles were experienced. First is that the target bacteria and conjugates were incubated at 37˚C and 4˚C before soaking the conjugate pad. Then, they were dried. Second is that the conjugate pad was prepared by soaking the conjugate without target and dried before applying the target. The biotinylated oligonucleotides on the test and control lines were immobilized, manually using micropipette. Detector aptamers were used as test lines while biotin modified complementary oligonucleotides against to the aptamers on the surface of AuNPs as

control lines. For this purpose, streptavidin was added into the bio-modified oligonucleotides, separately and then centrifuged at 14000 rpm. Remained solution was suspended in PBS and stored at -20˚C for the preparation of strip assays. The strips were prepared as duplicate and washed with either PBS or saline-sodium citrate buffer if needed and then results were analyzed with naked eye. The assay principle of the prepared LFAs was illustrated in Figure 1.

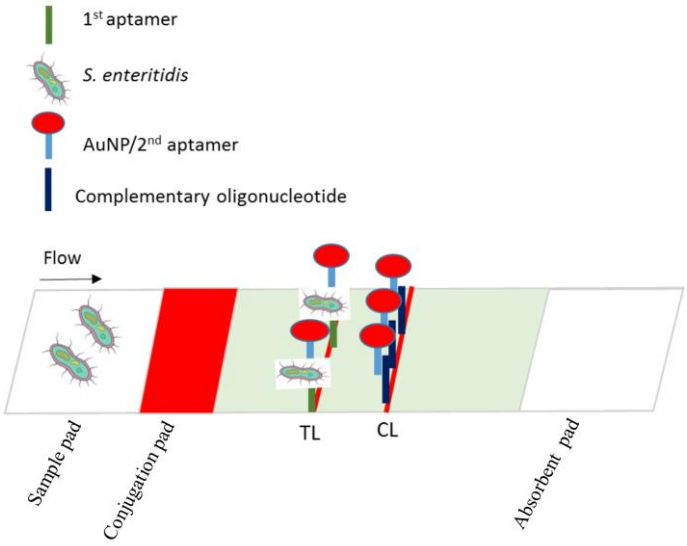

Figure 1. The working principle of the aptamer pairs based LFAs

3. Results

3.1. Synthesis and conjugation of gold nanoparticles

Figure 2 shows the AuNPs and conjugations. For the conjugation, 2µM and 4µM aptamer concentrations were found as sufficient for coating the AuNPs while 0.2µM aptamer was not enough for preventing the nanoparticles from aggregation (data not shown). To highlight, gold conjugate showed high stability when added with the target, *S. enteritidis*, while it was flocculated with *E. coli*, which means that aptamers covalently bound to AuNPs captured the target and continued the stability of conjugate (data not shown). Red color intensity of the conjugates also verified the aptamer binding to AuNPs since the negative control without aptamer showed aggregation. Maximum absorbance value of the surface plasmons (λ_{max}) of AuNPs was measured by NanoDrop 2000c Spectrophotometer (data not shown) and EPOCH2 Plate Reader/Spectrophotometer. Results were in consistent and λ_{max} was measured as 520±2nm for the naked AuNPs (Figure 2A, 1-2). The peak shift was observed as 526-530 nm for both conjugates (conjugate 1 and 2) as expected since the accumulation of aptamers on to AuNPs (Figure 2, 3-4).

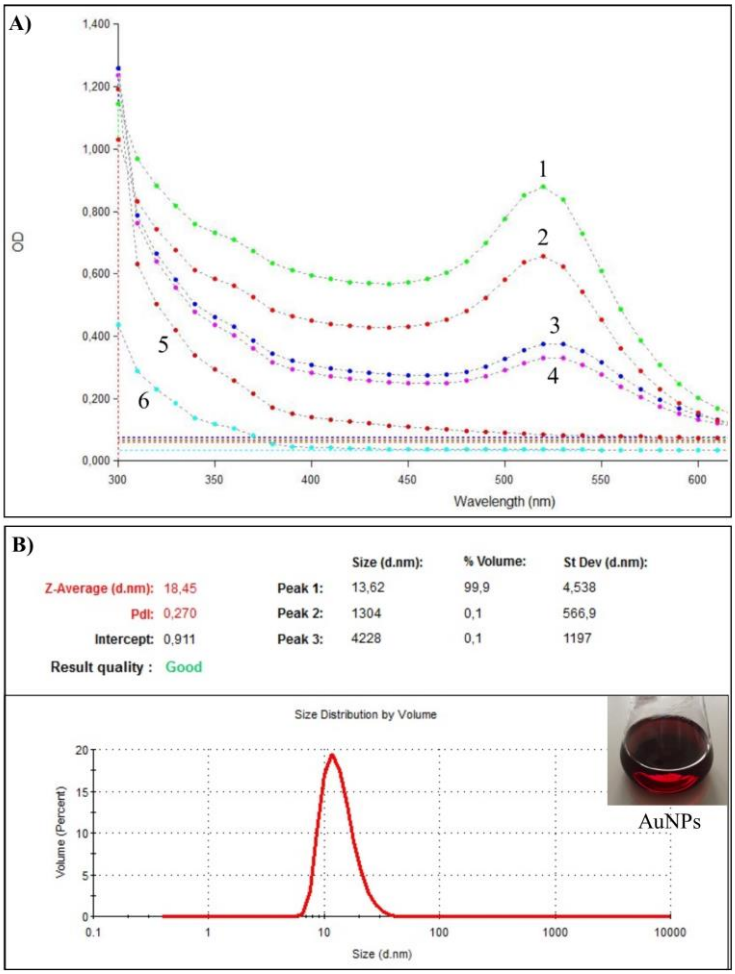

Figure 2. UV-Vis Spectroscopy and the size measurement of AuNPs. A) λ_{max} of naked (1-2) and conjugated AuNPs (3-4) Conjugate 2 and 1, respectively. 5: λ_{max} measurement of resuspension buffer. 6: λ_{max} measurement of dH2O. Bottom flat: AuNPs/aptamer solution without TCEP. The inset shows the color intensity and stability of naked nanoparticles

The size of the synthesized AuNPs were found as 18 nm (Figure 2B) and the Zeta Potential was -42 ± 1. (data not shown) while they were 35±1 nm and -11±2 for the conjugation (data not shown). After the accumulation of aptamers on AuNPs, both the size increment was observed and surface charge was shifted to less negative as expected. Because the reagents such as BSA or aptamers in suspension buffer not only cause to increasing of particle size but also change the surface charge.

3.2. Preparation of the strip tests

Two conjugations and four aptamer pairs were prepared and experienced for sandwich assay. Among the three buffer for sample pad soaking, buffer 3 was used for further experiments as it allowed to efficient flow and decreased the non specific binding. Among the four RBs, RB 1 and RB 14 did not show the non specific binding on test lines which were designed by 1st and 4th aptamer pairs (data not shown) and they were preferred for further experiments because of having a good assay performance. Control lines of all strips were visible, clearly which is meaning that tests are working and valid. Strip assays were also designed without conjugate pad, but both the conjugate and target were retarded on the pad and flow was not observed (data not shown). It is clearly evidence that conjugate pad is crucial for effective interaction between the gold conjugate and target, and also efficient flow through the membranes. Therefore, all the strip tests were prepared with conjugate pad for further experiments and results demonstrated that aptamer pairs could be usable in these conditions by applying the target bacteria.

3.3. Application of *S. enteritidis* to the prepared strip tests

The assay performance of the membranes having different flow time was compared with the aptamer pairs by applying the *S. enteritidis*. Figure 3 demonstrates the different membranes and buffer relations with two ways of target application into the assay. 1st way is to load the target into sample pad, directly (Figure 3A). 2nd way is to load the mixture of target and conjugate to the conjugate pad after incubating for 15 minutes (Figure 3B). According to the findings, M180 showed positive results with 1st pair using both RBs as the detection of *S. enteritidis* was occurred in the test lines, clearly (Figure 3, A1-A4) even if only one strip had weak test line intensity

(A2). Although the *S. enteritidis* was captured on test lines with all the aptamer pairs, a few of them did not showed the control line (Figure 3B, strip 2,4,7,8,9,11) using M120 membrane. Thus, it was decided that the best recognition of *S. enteritidis* was achieved by 1st aptamer pairs on M120 membrane (Figure 3B, strip 3,5,6) as it was similar with M180 membrane. However, the control lines of the strip 3 had weak line intensity. Generally, the weak line intensity on the control lines can be caused by the insufficient flow of conjugate and resulted with the weak interaction between the conjugate and complementary oligonucleotide. This could be understood from the Figure 3A, strip 1-4 and 5-6 because of the efficient flow on M180 membrane using the same aptamer pairs. Although the 4th aptamer pairs showed positive detection in M120 membrane, similarly the line intensity of control line was weak (Figure 3B, strip 10), but there was no detection on M180 membrane (Figure 3A, strip 5,6). This finding demonstrated the significance of the membrane and its suitability for used aptamer pairs. M240 membrane having a slow flow rate did not show the positive detection in these conditions mentioned above (data not shown). To highlight that the amount of conjugate has also significant role on line intensities. For instance, diluted conjugate may have weak color intensity compared to the non-diluted conjugate. This is reasonable because diluted conjugate means that aptamer concentration is low compared to the non diluted one. Thus, the number of captured target is low and accumulation of the conjugate after the recognition on test zones become weak. However, non diluted conjugate may also retard the flow (Figure 3B). As a result, 1st aptamer pairs were seen suitable for development of the strip assay compared to the other pairs and M180 was decided as the best membrane for further studies.

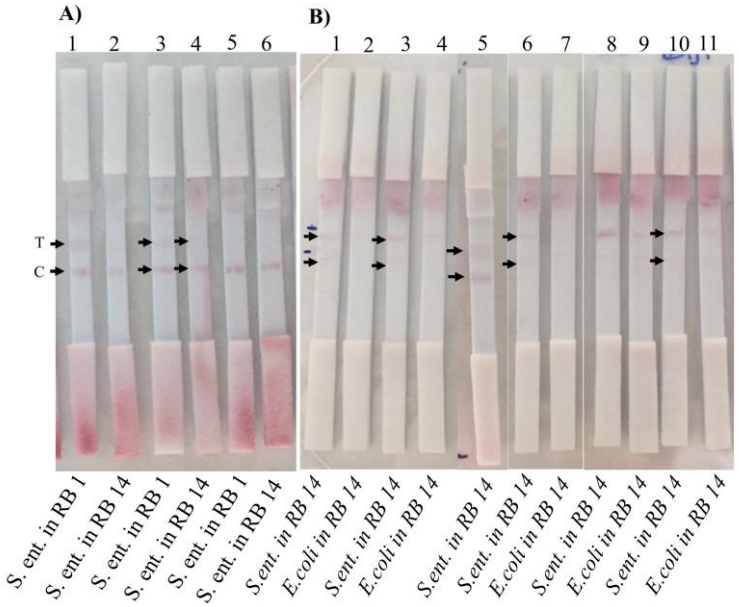

Figure 3. Application of *S. enteritidis* in different RBs to the LFAs prepared by M180 membrane (A) and M120 membrane (B) using 2µM conjugates. A) Strip 1-4: 1st pair, Strip 5-6: 4th pair. B) Strip 1-2: 3rd pair, Strip 3-7: 1st pair, Strip 8-9: 2nd pair, Strip 10-11: 4th pair. T: Test line C: Control line

In order to determine the optimal binding temperatures of aptamer pairs in the prepared assay, M180 membrane, buffer 3 as a sample pad buffer and RB 14 were also experienced here using the 4 µM conjugate. According to the results, 1st aptamer pairs showed the positive detection without non specific binding with the negative controls after the incubation of target bacteria and conjugate at 37˚C for 15 minutes (Figure 4, A1-A3). Although 2nd aptamer pairs recognized the target bacteria, non specific binding with the negative controls was observed (Figure 4, A6-A8). Interestingly, 3rd aptamer pairs could not detect the target bacteria and showed nonspecific binding with *E.coli*, weakly (Figure 4, A4-A5). This might be explained that the binding of the aptamer might be changeable and it can cause non specific interactions when the same aptamer is used as both the capture and detector aptamer in the sandwich assay. Besides, when the aptamer presenting on AuNPs binds to the target it could not be captured on the test line by the same aptamer as the binding site was already occupied. The similar result was also observed with 4th aptamer pairs since the same aptamer was used as detection and capture agent (Figure 4, A9-A10). Therefore, it might be inferred from that these aptamers should be used as a separate pairs for developing the point of care tests. Figure 4B also shows the results performed at 4˚C and 37˚C for 1 hour incubation of the target and conjugate. Results demonstrated that temperature is crucial for effective binding of these aptamer pairs to *S. enteritidis*. For instance, the recognition of *S. enteritidis* by the 1st aptamer pairs was better at 37˚C (Figure 4B, B4-B6) than 4˚C (Figure 4, B1-B3) in terms of the test line intensity. This was also verified and repeated by the previous results which were shown in Figure 4A, A1-A3 performed at 37˚C. Therefore, it can also be said that there is no significant difference between the 1 hour and 15 minutes incubation of the target and conjugate for the interaction between the aptamer and target bacteria. Interestingly, non specific interaction was occurred with the 1st aptamer

pairs by increasing the incubation time (data not shown). 2nd aptamer pairs showed non specific interaction with the negative controls (Figure 4A, A6-A8) at 37˚C. Although this problem was solved by changing the incubation time, the target was not detected this time (Figure 4B, B7-B12). This may be related with the time dependent binding potential of the aptamers and it needs further optimizations. Additionally, it also verified that the aptamer position on the sandwich assay has also critical role for effective recognition because the detection by the 1st aptamer pairs was successful compared to the 2nd aptamer pairs. To make a summary, 1st aptamer pairs seemed to have potential for developing LFAs at 37˚C with different incubation times (Figure 4, A1-A3; B4-B6). It was also seen that 2nd aptamer pairs have potential for preparing the LFAs after the incubation at 4˚C as the non specific interactions were not observed (Figure 4B, B7-B9). However, further optimizations for binding to target were needed. This might be possible with designing the new aptamer probes and capture sequences for the hybridization on sandwich assays.

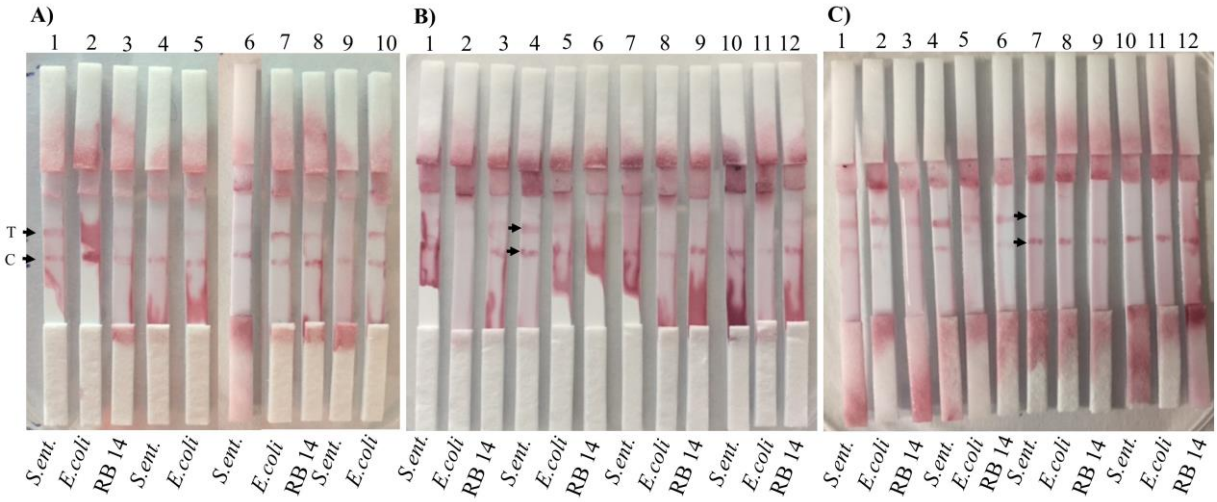

Figure 4. LFAs developed for deciding the optimum temperature of aptamer pairs for the recognition of *S. enteritidis* using 4 µM conugates. A) Strip 1-3: 1st pair, Strip 4-5: 3rd pair, Strip 6-8: 2nd pair, Strip 9-10: 4th pair. *S. enteritidis* and conjugate were incubated at 37˚C for 15 minutes. B) Strip 1-6: 1st pair, Strip 7-12: 2nd pair. *S. enteritidis* and conjugate were incubated at 4˚C (strip 1-3; strip 7-9) and 37˚C (strip 4-6; strip 10-12) for 1 hour

4. Discussion and Conclusion

Four aptamer pairs specific to *S. enteritidis* were applied to the prepared LFAs. To the best of our knowledge, this is the first study showing the applicability of these two aptamers to the strip assay by using as different aptamer pairs. Therefore, the assay optimizations including running buffer, membrane types having different flow rate, incubation time and temperatures and aptamer concentrations were experienced. Results showed the rapid detection of *S. enteritidis* without any further experimental steps such as magnetic separation (27), PCR (28) or preparing electrochemical biosensors based on aptamers (29). M180 membrane was found to be suitable in this study. Although 1st and 2nd aptamer pairs were found as suitable for developing the LFAs, 1st aptamer pairs were better than others in these conditions and 2nd aptamer pairs needs to further optimizations for efficient detection. Aptamer pair based LFAs also showed some critical issues for developing the strip assay for the usage of *S. enteritidis* detection. First is the design of aptamer pairs. If the pairs used for the recognition and capture are used separately, target recognition is better than the usage of the same aptamer as a recognition and capture agent. This is reasonable because different aptamers bind to the different surface of target resulted by efficient detection. However, aptamer presents on the test line may not capture the target, efficiently since the surface of target is already occupied by itself when the same aptamer is present on the AuNPs. This was observed by using 3rd and 4th aptamer pairs in this study. Second is the aptamer and conjugate concentrations. If the aptamer is not enough for coating the AuNPs it causes agglomeration and capture lines can not be visible with the low amount of conjugate. Third is the assays buffers. The aptamer binding is affected by the temperature and buffers. To obtain the positive results, temperature and buffers specific to aptamers should be optimized and used for the efficient binding. Fourth might be an aptamer length. Although aptamers having primer sequences were used here, the form of aptamers without primer regions were not experienced. Thus, since the primer regions may cause hairpin and affect the binding of aptamers to the target the non specific interactions might be occurred here and this will be considered as a further study. As a summary, two candidate aptamers for developing the LFAs were examined here, and it was concluded that these aptamers could be used as pairs and adopted to the strip assay for the rapid detection of *S. enteritidis*.

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