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A rapid DNA biosensor for the molecular diagnosis of infectious disease

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ABSTRACT

Treating patients with infectious diseases relies heavily on rapid and proper diagnosis. Molecular detection such as PCR has become increasingly important and efforts have been made to simplify these detection methods. This study reports the development of a glass fibre-based lateral flow DNA biosensor that uses capture reagents coupled to carrier beads and detector reagent bioconjugated to gold nanoparticles, for the detection of foodborne pathogen, *Vibrio cholerae*. The DNA biosensor contains a test line which captures target PCR amplicons, an internal amplification control (IC) line which captures IC amplicons and a control line which acts as membrane control to validate the functionality of this device. The test line captures biotin labelled DNA, while the IC line captures digoxigenin labelled DNA. The detector reagent recognizes the fluorescein haptens of the amplified DNA and produces visual red lines. Scanning electron microscopy (SEM) studies performed indicated that the capture reagents remained relatively immobile within the matrix of the membrane even after binding of the detector reagent. The DNA biosensor recorded a limit of detection (LoD) of 5 ng of target DNA. A clinical evaluation was carried out with 174 strains of *V. cholerae* and non *V. cholerae* bacteria and the DNA biosensor recorded 100% for both sensitivity and specificity when compared to conventional agarose gel detection of DNA. Thus it is a viable alternative to agarose gel analysis and is easy-to-use, disposable and do not require any specialized equipment and use of carcinogenic chemicals.

1. Introduction

Rapid and accurate detection of causative pathogen is essential in determining the choice of treatment in acute-care settings. Molecular diagnosis is an increasingly popular approach as it is highly sensitive, specific and has a faster turnaround time (Pitt and Saunders, 2000; Tang et al., 1997). Nucleic acid amplification-based methods provide better specificity through the use of a specific complementary sequence to the target. These methods also have higher sensitivities as it is possible to detect a theoretical single gene copy through the amplification process (Yager et al., 2008).

Several techniques have emerged for the amplification of nucleic acids, which include amplification of the hybridising probes such as ligase chain reaction Qβ replicase amplification (Lomeli et al., 1989; Schachter et al., 1994); transcription-based amplification such as transcription-mediated amplification and nucleic acid sequence-based amplification (Compton, 1991; Jungkind, 2001; Tang et al., 1997); and signal amplification such as branched DNA and hybrid capture (Sanchez-Pescador et al., 1988; Tang et al., 1997). However, the polymerase chain reaction (PCR) technique still remains the best developed and most widely used technique for DNA amplification, especially in molecular diagnosis, due to its relatively ease of use and reproducibility of results (Li and Tang, 2006; Tang et al., 1997; Yang and Rothman, 2004).

However, the majority of nucleic acid amplification based techniques still require analysis of the amplified products with either agarose gel electrophoresis or probe hybridization methods. These agarose gel detection procedures expose users to hazardous elements of ethidium bromide and ultraviolet light, while common probe based methods require specialized equipment and multiple hybridization and washing steps, which are labour-intensive and time-consuming. Newer methods such as electrochemical biosensors are reported to be very sensitive for detecting DNA or PCR amplicons of different pathogens (Aitichou et al., 2004; Liao et al., 2006; Yean et al., 2008). These biosensors usually operate on the principle of immobilization of a probe on the surface of the sensor, hybridization and detection by electrochemical (Ozsoz et al., 2003; Yean et al., 2008), optical (Mannelli et al., 2007), or gravimetric transduction (Tombelli et al., 2005). Various other sensitive and high-end label-free methods to detect DNA have been developed using capacitance measurement circuit (Guiducci et al., 2004) or biochips (Carrara et al., 2009) or morpholino-functionalized sil-

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icon nanowires (Zhang et al., 2010). However, these biosensors require instrumentation and, in most cases, involve several incubation and washing steps and thus are tedious for the detection of PCR amplicons.

Lateral flow devices have been increasingly used as alternative tools for detecting PCR amplicons (Baeumner et al., 2003, 2004; Horng et al., 2006; Kozwich et al., 2000; Suzuki et al., 2006). Advantages of the lateral flow platform include ease-of-use, disposable format, rapid, little training to perform and relatively low cost (Kumanan et al., 2009). However, most of these DNA lateral flow devices do not include PCR internal amplification control (IC) detection or provide membrane control as validation of device functionality (Dineva et al., 2005; Horng et al., 2006; Kalogianni et al., 2006; Vlachou et al., 2010). Moreover, nitrocellulose membranes were usually employed, requiring complex optimization of multiple materials for compatibility issues, complicated blocking strategies due to its hydrophobic nature and thus incurred high development cost and time (Jones, 2009). Probe based lateral flow DNA biosensors have also been reported for detecting *Trypanosoma cruzi* and *Leishmania* (Deborggraeve et al., 2009; Espinosa et al., 2009), but these tests required additional steps of heat denaturation of amplicons and pre-heating of running buffers. Studies using a combination of PCR and primer extension reaction (PEXT) were also done to amplify DNA for detection with lateral flow devices. These methods have been demonstrated for detecting single nucleotide polymorphism (SNP) and genetic diseases with better specificity (Kalogianni et al., 2009; Litos et al., 2007; Vlachou et al., 2010). Moreover novel labels such as up-converting phosphor reporters (UPT) and dye-entrapping liposomal nanovesicles (Baeumner et al., 2003, 2004; Corstjens et al., 2001) which have proven to increase the sensitivity levels, are still not popular because of their cost (Chun, 2009; Corstjens et al., 2003). In contrast, gold labels are known to work well for lateral flow devices, since they are stable in both liquid and dried forms and their colours do not fade after staining on membranes (Chun, 2009). Thus, gold nanoparticles were used as the detector labels of the DNA biosensor developed in this study.

On this basis, this study describes a complete in-house development of a universal lateral flow based DNA biosensor which incorporates 3 lines, each for target PCR amplicon detection, IC detection and membrane control. The DNA biosensor was developed for the detection of foodborne pathogen, *Vibrio cholerae*. Specific PCR primers were designed for *V. cholerae* with 5′ ends modified with hapten labels for specific recognition by the capture reagents as well as the detector reagents. IC detection helps to rule out the presence of inhibiting substances in a PCR. This system was then evaluated for its limit of detection (LoD) and tested with stool spiked clinical specimens.

2. Materials and methods

2.1. Chemicals and equipments

Gold nanoparticles (GNPs) of 40 nm in size were purchased from BBInternational (Cardiff, UK). Streptavidin-coated microspheres and carboxylate-modified polystyrene microspheres were purchased from Estapor (Fontenay sous Bois Cedex, France). Anti-fluorescein antibodies, anti-digoxigenin antibodies and goat anti-mouse IgG antibodies were ordered from Pierce Biotechnology (Rockford, USA), Roche Diagnostics (Penzberg, Germany) and Invitrogen (CA, USA) respectively. Micro BCA Protein Assay Kit (Pierce Biotechnology, IL, USA), FUSION 5 membranes were from Whatman (Kent, UK), absorbent pads were from Millipore (MA, USA), while plastic adhesive backing cards were from G&L Precision Die-cutting (Amstelveen, the Netherlands). GoTaq Flexi DNA Polymerase kit was obtained from Promega (WI, USA) while dNTP mix was from Fermentas (Vilnius, Lithuania).

Bovine serum albumin (BSA) was purchased from Amresco (OH, USA), while 2-(N-morpholino) ethanesulfonic acid (MES) was from Merck (Darmstadt, Germany). Ethyl-3,3(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC), ethanolamine, sodium azide (NaN_3) and all other common chemicals were from Sigma (MO, USA).

The DNA biosensor was lined with reagents using an XYZ3050 (BioDot, CA, USA) dispensing platform and cut into strips using a Kinematic Automation (CA, USA) automatic strip cutter. PCR amplification reactions were performed using a Bio-Rad DNA Engine (CA, USA) thermal cycler and the amplicons analysed using a Chemi-Imager 5500 (Alpha Innotech, CA, USA) UV illuminator and image capturing unit.

All labelled oligonucleotides were synthesized by First BASE (Selangor, Malaysia) with the following sequences: target forward primer (*lol*B-F), 5′-biotin-GTG CAT CTT GGT CGC GGT AG -3′; target reverse primer (*lol*B-R), 5′-fluorescein-GGC AAT CAC ACC AAG TCA CTC-3′; internal amplification control forward primer (IC-F), 5′-digoxigenin-GGT ACC TTT ACC ACA AGT TAC AC-3′; and internal amplification control reverse primer (IC-R), 5′-fluorescein-TTC TCG ATC GTC TTC TGG AT-3′. These primers were designed and its sensitivity and specificity evaluated in a previous study (Chua et al., 2010).

2.2. Bioconjugation of antibodies to gold nanoparticles (GNPs)

GNPs were bioconjugated with anti-fluorescein antibodies to produce detector reagents for the DNA biosensor for visual detection. Conditions for bioconjugation were optimized using salt flocculation technique (Hermanson, 1996; Paek et al., 2000; Xiulan et al., 2005) whereby the gold conjugates formed at different pH and antibody concentrations were challenged with ionic salts to determine stability of the conjugates. With the optimal conditions, 40 μg of anti-fluorescein antibody in 4 ml of 2 mM borax buffer was added drop-wise to 20 ml of GNP solution, pH 8.0 while stirring. The mixture was then incubated at ambient room temperature with end-to-end mixing for 30 min. The conjugates were blocked by adding 1 ml of 25% (w/v) BSA solution to achieve a final 1% (w/v) BSA blocking mixture. The mixture was centrifuged at 10,000 × *g* for 15 min to remove unconjugated antibodies from the solution. The pellet obtained was dispersed in 2 ml of 2 mM phosphate buffer, pH 7.4, 1% (w/v) BSA, 0.1% (w/v) NaN_3 to achieve a stock conjugate solution of OD_{530} = 20.0 by measuring at a 1:100 dilution factor. The stock detector reagent was stored at 4–8 °C for further experiments.

2.3. Coupling of antibodies to microspheres

Antibodies coupled to microspheres were used as capture reagents for the DNA biosensor. Anti-digoxigenin antibodies and goat anti-mouse IgG were covalently coupled to carboxylate-modified polystyrene microspheres using a modified protocol from Polysciences (2007) while streptavidin-coated microspheres were obtained commercially. Briefly, microspheres (12.5 mg) were washed with 0.1 M MES buffer pH 6.0 and resuspended with 625 μl of the same buffer. A freshly prepared 2% EDC solution in 625 μl was added to activate the microspheres. The activated microspheres were washed once in 1.2 ml of 0.1 M MES buffer, followed by another wash with 1.2 ml of 0.2 M borate buffer, pH 8.5. The activated microspheres were then resuspended in 600 μl of 0.2 M borate buffer, pH 8.5. Anti-digoxigenin antibody (0.33 μg/μl) and goat anti-mouse IgG antibody (0.50 μg/μl) in 600 μl of 0.2 M borate buffer were added and incubated for 2 h. The mixture was then centrifuged at 10,000 × *g* for 15 min and the supernatant was analysed for protein using Micro BCA Protein Assay Kit to determine the cou-

pling efficiency. The antibody-coupled microspheres were blocked twice using 1.25 ml of 0.01 M ethanolamine and 1 ml of 10 mg/ml BSA in 0.2 M borate buffer. The mixture was finally resuspended in 1.25 ml of 0.01 M phosphate buffer, pH 7.4 with 0.1% (w/v) BSA, 0.05% (w/v) NaN_3 and stored at 4–8 °C until further use.

2.4. Construction of the lateral-flow DNA biosensor

The lateral flow dipstick (5 mm × 72 mm), depicted in Fig. 1, consisted of a conjugate area (14 mm) and a sample application area cum reaction area (32 mm) constructed from FUSION 5 membrane with an absorbent pad (30 mm) assembled on a plastic adhesive backing card. The capture reagents were dispensed onto the reaction area as the test line, IC line and control line, with each line separated by 5 mm, using the XYZ3050 dispensing system equipped with Front Line dispensers. The optimal amount for each capture reagent was determined empirically and visually evaluated for line intensity using a mixture of positive PCR amplicons and detector reagents. The detector reagents were sprayed onto the conjugate area in the presence of a mixture of protein blocker and sugar stabilizers using the XYZ3050 dispensing module equipped with an AirJet dispenser at 3 psi. Strips of the assembled membrane were then cut at 5 mm widths with the automatic strip cutter. The DNA biosensor strips were stored in an aluminium pouch with desiccants at room temperature until use.

2.5. Visual detection of PCR amplicons using the DNA biosensor

A volume of 10 μl of PCR amplicons was added to the sample application area of the biosensor. The strip was then immersed into 50 μl of running buffer (10 mM PBS, pH 7.4 with 0.5% Tween-20) followed by another 100 μl of the same buffer. The dipstick was allowed to absorb the entire running buffer. After 10 min, the DNA biosensor was observed for the presence of coloured lines. The presence of all 3 red coloured lines namely the test line, IC line and control line, on the biosensor indicates a valid positive result, while the presence of coloured lines only in the IC line and control line denotes a valid negative PCR assay. The control line should always be present to confirm the proper function of the DNA biosensor.

2.6. Scanning electron microscopy analysis

Scanning electron microscopy (SEM) was performed using a Leo Supra 50 VP Field Emission scanning electron microscope (Zeiss, Göttingen, Germany) to analyse the immobilization of capture reagents on the DNA biosensor. Sections of the capture reagent zones before and after the binding of the PCR product and detector reagent were prepared and viewed under variable pressure SEM with 5000× magnifications.

2.7. Cholera PCR protocol

A PCR assay for *V. cholerae* was carried out in a final volume of 20 μl containing 1× Green GoTaq Flexi Buffer, 1.5 mM $MgCl_2$, 0.16 mM of each of the dNTPs, 0.6 pmol/l of each hapten labelled primers for the target gene, 0.2 pmol/l of each primers for IC, and 1 U of GoTaq DNA polymerase. In addition, IC target plasmids (40 pg) were incorporated into the mixture and serves as template for the IC. Target primers were expected to amplify the target *lol*B gene of *V. cholerae* (Lalitha et al., 2008) to produce amplicons of 237 bp in size, while the amplification of IC was expected to generate 150 bp amplicons. IC was utilized to rule out false-negative results of a PCR assay due to inhibiting substances (Reiss and Rutz, 1999).

PCR was performed in a thermal cycler with one initial denaturation step at 95 °C for 2 min, 35 cycles of denaturation at 95 °C for 30 s, annealing 60 °C for 30 s, and extension at 72 °C for 30 s, followed by an extra annealing step of 65 °C for 30 s and a final extension step at 72 °C for 5 min. The amplification products were resolved in 2.0% agarose gel electrophoresis, stained with ethidium bromide, visualized under UV illumination and photographed using an image analyzer.

2.8. Evaluation of the DNA biosensor

The DNA biosensor was evaluated with a panel of 174 bacteria spiked into healthy stool specimens to mimic clinical stool specimens. The bacteria consist of 102 *V. cholerae* strains and 72 other non *V. cholerae* strains comprising different Gram positive and Gram negative bacteria. Briefly, stool suspensions were prepared based on a modified protocol from McOrist et al. (2002) and spiked with a single colony of bacteria. The spiked stool specimens were then inoculated into 5 ml of APW and incubated for 6 h. Then, 1 ml of the culture was boiled and centrifuged. The supernatant was used as template for cholera PCR. The resultant amplicons were then tested with the DNA biosensor and confirmed with agarose gel analysis.

The LoD of the DNA biosensor was analysed using PCR amplicons from a known *V. cholerae* strain. The PCR products of both *lol*B and IC genes were purified using PCR Clean-Up System kit (Promega, WI, USA) and quantified using a Biophotometer spectrophotometer (Eppendorf, Hamburg, Germany). The two purified PCR products were mixed into a single tube with equimolar concentrations of each PCR product to obtain the concentrations of 10.0, 5.0, 1.0, 0.5 and 0.1 ng/μl. A 10 μl aliquot from these different concentrations of equimolar PCR products was tested using the DNA biosensor and the results were subsequently compared with agarose gel analysis.

3. Results and discussion

3.1. Detection of PCR amplicons

A schematic illustration of the principle of the DNA biosensor for visualization of PCR amplicons is presented in Fig. 1. The DNA biosensor detects PCR amplicons through specific recognition of the hapten labels on the 5′ ends of amplicons, which were generated by using hapten labelled primers. Amplicons from the target *lol*B gene of *V. cholerae* were biotin and fluorescein labelled, whereas the IC amplicons were digoxigenin and fluorescein labelled. The PCR sample is deposited onto the sample application zone of the DNA biosensor, which is then immersed in the running buffer. The buffer migrates along the strip by capillary action and rehydrates the immobilized detector reagents (anti-fluorescein antibody conjugated gold nanoparticles). The target amplicon is captured at the test line through biotin/streptavidin interaction, while the IC amplicon is captured at the IC line through specific antibody recognition of digoxigenin labels of the amplicon. Detector reagent then accumulates on the test line and IC line due to specific recognition of the fluorescein haptens of the captured amplicons. Accumulation of the detector reagent will result in a visual red coloured line in the reaction area of the DNA biosensor. The proper functioning of the DNA biosensor is confirmed by the control line formation which contained immobilized goat anti-mouse IgG antibody-coated microspheres that capture excess detector reagent.

3.2. Optimizing antibody conjugation to GNP and microspheres

The pH conditions and minimal antibody amounts needed to produce stable gold conjugates were optimized using salt flocculation studies. The optimal pH was between pH 6 and 11. The pH value of 8 was chosen as it was the median value of the acquired pH range, and was similar to a previous study (Xiulan et al., 2005). The

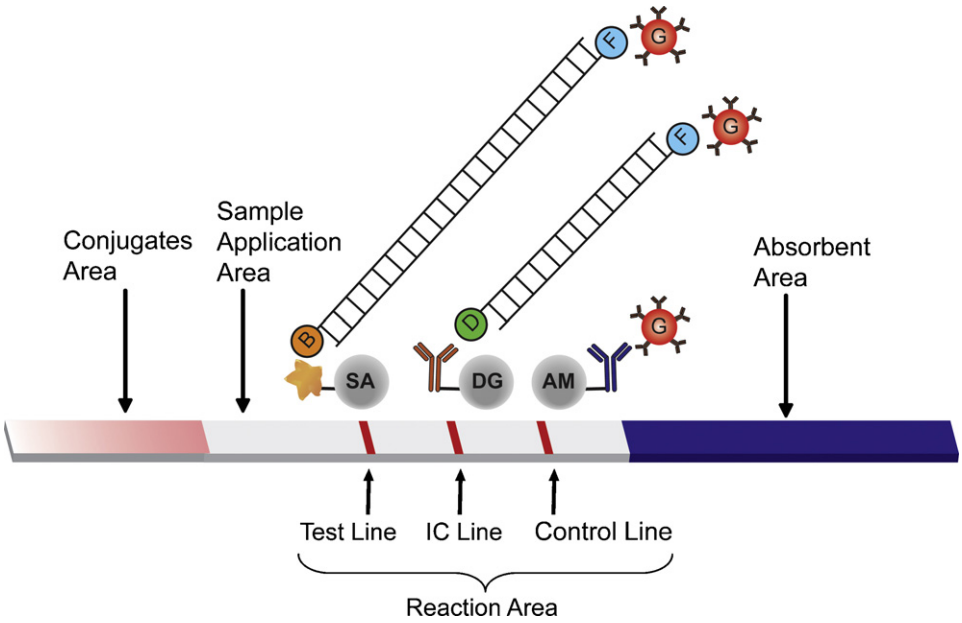

Fig. 1. Schematic illustration of the principle of the DNA biosensor for visualization of PCR amplicons by using capture reagents coated onto carrier beads and immobilized onto a glass fibre membrane. The target PCR amplicon is biotin and fluorescein labelled and is captured on the test line, in while IC amplicon is digoxigenin and fluorescein labelled and is captured on the IC line. Accumulation of detector reagent by specific recognition of the fluorescein haptens produces a visual signal. Excess detector reagent is captured on the control line and validates the working condition of the biosensor. SA = streptavidin-coated microspheres; B = biotin hapten; F = fluorescein hapten; DG = anti-digoxigenin antibody-coated microspheres; D = digoxigenin hapten; AM = goat anti-mouse IgG antibody-coated microspheres; G = anti-fluorescein antibody conjugated gold nanoparticles.

minimal antibody amount was determined to be at 2 μg for every 1 ml of pH optimized GNPs. GNPs were used as the detector labels for the DNA biosensor as gold conjugates are known to maintain stability in both liquid and dried forms (Chun, 2009). The 40 nm sized ones were chosen as they are red at visible wavelength and offer maximum visibility with the least steric hindrance (Wong and Tse, 2005).

Covalent coupling of antibodies to microspheres was performed using EDC chemistry. The coupling efficiency was determined by measuring the amount of protein (antibody) present in the supernatant before and after covalent coupling. The amount of protein left in the supernatant was substantially less after the coupling step (data not shown) thus proving that antibodies were successfully coupled to the microspheres. However it was observed that the coupling efficiency for anti-digoxigenin antibody was higher than goat anti-mouse IgG antibody. It may be due to the different host sources of the antibodies having different coupling affinities.

3.3. Construction of the DNA biosensor

The amount of capture reagents to be lined was first investigated in order to ensure the best line intensity from the lowest amount of capture reagents. Different amounts of capture reagents from 20 to 80 μg were deposited in different DNA biosensor strips and tested with 10 μl of a positive PCR sample and detected with the detector reagent. The red coloured lines formed were visually evaluated and given a score (Table 1). The minimal amount of capture reagent to produce intense red lines was 20 μg for both streptavidin-coated microspheres and anti-digoxigenin antibody-coated microspheres, while goat anti-mouse IgG antibody-coated microspheres required up to 60 μg per biosensor strip. The optimal amount for streptavidin-coated microspheres and anti-digoxigenin antibody-coated microspheres was similar to the product sample protocol given by the manufacturer (Whatman, 2005). However, the higher amount needed for goat anti-mouse IgG antibody-coated microspheres could be attributed to a less efficient covalent coupling of the antibody to polystyrene microparticles. Based on these results, the optimized DNA biosensor was constructed.

As shown in Fig. 1, the DNA biosensor was constructed with the conjugate area as the first zone during migration of the running buffer, followed by the sample application area and the reaction area. The wicking pad is placed last to absorb excess running buffer and detector reagents. The sample application area was designed to be downstream of the conjugate area, similar to previous reports (Kalogianni et al., 2006, 2007). This is because the DNA sample volume to be tested is relatively small compared to the water absorption value of the FUSION 5 membrane. This design allowed the DNA sample to reach the reaction area first before the rehydrated detector reagents migrate along with running buffer.

The DNA biosensor here utilizes a unique glass fibre membrane known as FUSION 5 as a substitute for nitrocellulose membrane and uses capture reagents coupled to carrier beads and detector reagent bioconjugated to gold nanoparticles. FUSION 5 was used as the conjugate pad, sample application pad and the reaction pad in this lateral flow-based DNA biosensor. The capture reagents were coated onto polystyrene microparticles carrier beads which served as anchors that immobilize the capture reagents within the matrix of the membrane. A similar scheme named as the rapid immunofilter paper assay (RIPA) was developed for the detection of plant virus in which antibody-coated latex beads were used to detect viral antigens (Tsuda et al., 1992). Yet, till now no similar device was developed to detect DNA using this principle.

Scanning electron microscopy (SEM) studies was carried out to study the immobilization of the capture reagent in the membrane. The study revealed that the capture reagents remained relatively stationary before and after the binding of the PCR product and the detector reagent to the test line (Fig. 2). This showed that the immobilization of capture reagents was quite stable even after the passage of liquid through the membrane. This could be due to the hydrophilic nature of the membrane (Jones, 2009), which also has

Table 1
Visual evaluation of line intensity with different amounts of capture reagents.

Amount of capture reagent dispensed per strip	Capture reagent			
	20 μg	40 μg	60 μg	80 μg
Streptavidin-coated microspheres	+++	+++	+++	+++
Anti-digoxigenin antibody-coated microspheres	+++	+++	+++	+++
Goat anti-mouse IgG antibody-coated microspheres	+	++	+++	+++

+++ = very intense red line; ++ = intense red line; + = less intense red line.

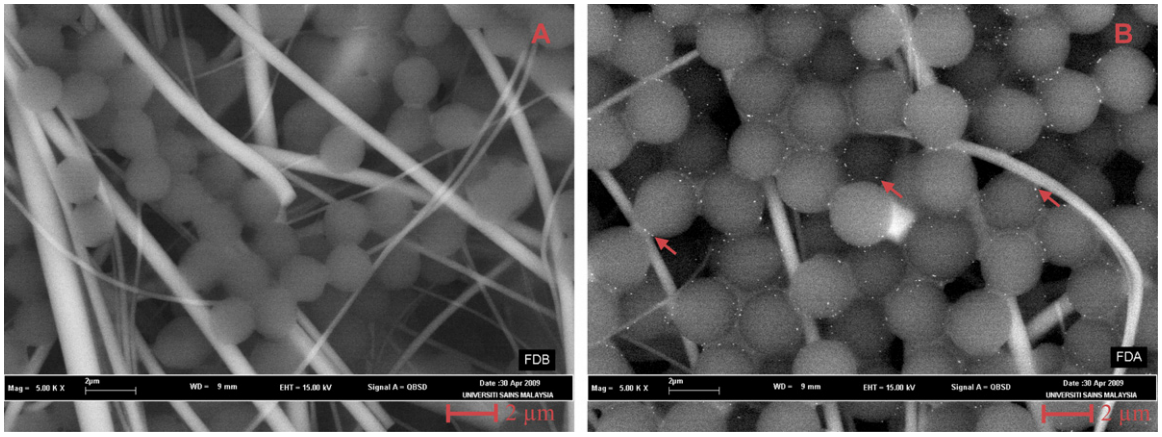

Fig. 2. Representative images of the scanning electron microscopic study on the immobilization of capture reagents within FUSION 5 membrane. Both images showed the 5000× magnification of the capture reagent zone before (A) and after (B) the binding of PCR product and detector reagent. Red arrows indicate the accumulation of detector reagents on the surface of capture reagents after the passage of running buffer through the DNA biosensor.

the advantage of not requiring any blocking procedure after dispensing of capture reagents.

The stability or shelf life of the DNA biosensor was also studied by performing accelerated aging techniques based on elevated temperatures, known as the Q_{10} method. This technique is based on measuring storage of a medical device in elevated temperatures for a shorter period of time and mathematically correlating increased temperature with time (Clark, 1991). The DNA biosensor strips, stored at 37 °C, maintained its activity up to 30 days. This translates to an estimated shelf life of 103.87 days at ambient temperature of 24 °C. This suggests that the DNA biosensor can be transported and stored without cold chain to remote areas and thus can be performed and interpreted easily by less skilled personnel.

3.4. LoD of the DNA biosensor

The LoD of the DNA biosensor was determined by comparing the results obtained with the conventional agarose gel method when varying amounts of PCR amplicons. It was observed that the DNA biosensor showed clear visible red lines for both test line and IC line when the DNA concentration was as low as 5 ng while the agarose gel showed very faint bands at the same concentration (Fig. 3). This suggests that DNA biosensor can provide almost the same sensitivity as the conventional agarose gel while not involving the use of carcinogenic chemicals. Previous studies have reported DNA biosensors with better LoD (Kalogianni et al., 2006, 2007), but the detection procedure required an additional hybridization step.

3.5. Detection of clinical specimens

The specificity of the DNA biosensor was evaluated using 174 bacteria-spiked stool specimens consisting of various types of *V. cholerae* strains and other enteric bacteria. PCR was performed using these specimens and the amplicons were detected using the DNA biosensor. Agarose gel detection method was used as the gold

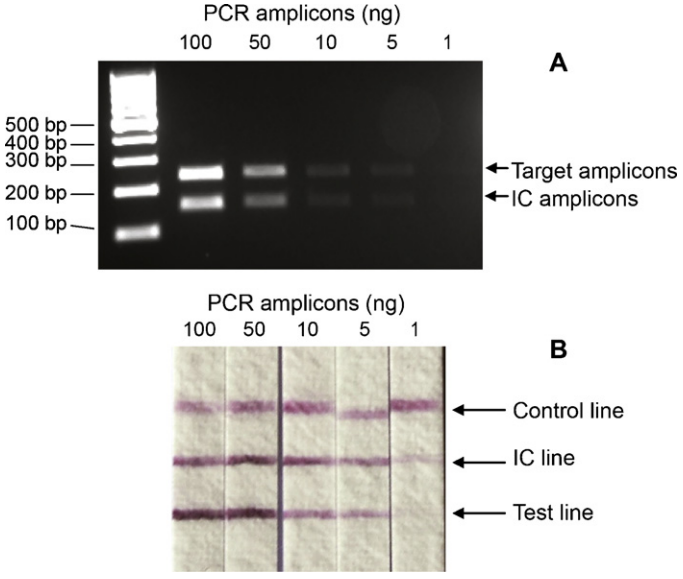

Fig. 3. The limit of detection (LoD) of the DNA biosensor was determined using different concentrations of PCR amplicons ranging from 1 to 100 ng. (A) shows LoD of agarose gel method as 5 ng and (B) shows the LoD of the DNA biosensor as 5 ng. M = DNA markers.

standard to evaluate the efficiency of the DNA biosensor results. A comparison of the results obtained with the DNA biosensor and agarose gel is presented in Fig. 4. The DNA biosensor results were in concordance with agarose gel analysis results. It showed 100% sensitivity and specificity when compared to agarose gel analysis as the gold standard. The summary of the DNA biosensor results with 174 samples is shown in Table 2. These results proved that the DNA biosensor performed in perfect similarity to the conventional agarose gel method.

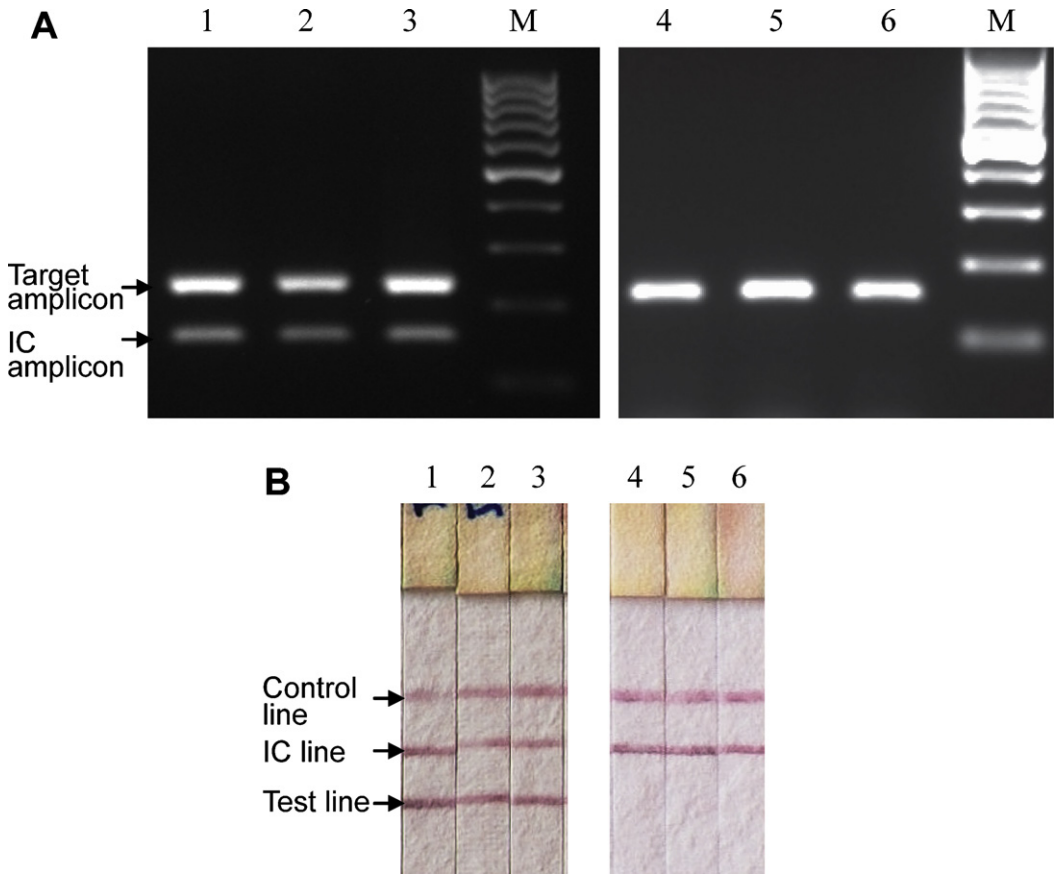

Fig. 4. A representation image of the agarose gel analysis results and DNA biosensor results obtained using clinical specimens is shown here. PCR was performed on the clinical specimens. A 10 μl aliquot of the PCR sample was detected using 2% agarose gel electrophoresis and ethidium bromide stained (A) and with the DNA biosensor (B). M = DNA marker; Lanes 1–3 = PCR product from *V. cholerae* samples; Lanes 4–6 = PCR product from non-*V. cholerae* samples.

Table 2
Summary of the results of DNA biosensor and agarose gel analysis obtained using clinical specimens.

Strains (n = 174)	DNA biosensor results		Agarose gel results	
	Target gene (*lol*B)	Internal control (IC)	Target gene (*lol*B)	Internal control (IC)
V. cholerae				
Serogroup O1 (n = 86)	86	86	86	86
Serogroup O139 (n = 12)	12	12	12	12
Serogroups non-O1/non-O139 (n = 4)	4	4	4	4
Other bacteria				
Other *Vibrio* spp. (n = 5)	0	5	0	5
Gram positive bacteria (n = 31)	0	31	0	31
Gram negative bacteria (n = 36)	0	36	0	36

Sensitivity and specificity: 100%. n = number of strains.

4. Conclusion

This study reported the development of a novel disposable lateral flow-based DNA biosensor that is highly specific and sensitive for the detection of PCR amplicons and is based on a unique glass fibre membrane. The biosensor uses a glass fibre membrane-carrier bead system, often described as the "boulders-in-a-stream" approach (Bangs, 2002), in which capture reagents are immobilized onto a hydrophilic membrane by using carrier beads which anchors the capture reagents permanently and allows the passage of liquid through without much hindrance. This approach has provided a suitable platform that avoids background signal problems while maintaining a relative constant flow rate of liquids along the strip. The DNA biosensor was demonstrated to be better than the conventional agarose gel electrophoresis as it is easy to perform, does not require any equipment for visualization of results, it is disposable and provides better sensitivity. The DNA biosensor was developed for the direct detection of labelled PCR amplicons without any post-hybridization procedure. But the DNA biosensor has its own limitation as the results are visualized qualitatively as red coloured lines. The aim of this study was to develop a faster and simpler alternative to agarose gel electrophoresis for analysing PCR amplicons. Thus the DNA biosensor developed can improve the utility of PCR as a diagnostic tool. PCR diagnosis is more widely implemented especially for diseases such as cholera, typhoid and parasitic infections affecting the developing and under-developed countries.

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