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DNAFoil, a novel technology for the rapid detection of food pathogens: Preliminary validation on *Salmonella* and *Listeria monocytogenes*

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ORIGINAL ARTICLE

Abstract

Over the past decades, several tools have been developed for food pathogen detection, including proteomics, metabolomics, immunological, biosensor, and nucleic acid-based approaches. Although these techniques are reliable and precise, they are time-consuming, technically challenging, and costly. Hence, it is necessary to develop rapid techniques for food pathogen detection, which can be performed at the household level. DNAFoil mechanism is a portable, completely self-administered, on-site DNA test that does not need expensive instruments or settings to confirm food pathogen detection in as little as 30 min. DNAFoil was used successfully for detecting food contamination and adulteration with pork derivatives (down to 0.1%) and vegetal components (down to 0.01%), respectively. In this study, initial validation experiments of DNAFoil were investigated to detect *Listeria monocytogenes* and *Salmonella* contamination. To confirm the specificity of the proposed method toward *Salmonella*, 18 different *Salmonella* strains, 6 non-*Salmonella* bacteria, and 2 fungi were investigated; also, in the case of *Listeria monocytogenes*, five bacterial strains, two fungi, and *Listeria monocytogenes* were investigated. The results stated that the Swiss Decode *Salmonella* and *L. monocytogenes* solutions can detect as few as 1 and 10 copies of DNA per microliter, respectively. The results also showed that the accuracy of our method ranges between 92 and 100%, while the precision value ranged between 88 and 100%. In terms of quality control applicability, DNAFoil *Salmonella* and *Listeria monocytogenes* reactions could be visually detected with the naked eye using a lateral flow strip, which could be used for in-place quality control during manufacturing and also can be used for more lab tests. In terms of cost, DNAFoil is usually much cheaper than the traditional detection methods. Therefore, DNAFoil is proposed as a promising and universal detection technology for food pathogens.

Keywords: DNAFoil technology; food pathogen detection techniques; food safety; foodborne diseases; health and economics threats

Introduction

It is well-known that food safety is affected by many factors and variables, including, for example, globalization of food trade, population increase in the world, change in the lifestyle of consumers, climate changes, and the accompanying rapid changes in food systems. International trade means that unsafe food can be distributed widely (Pinu, 2016; WHO, 2020). Recently, many food-borne disease outbreaks in the world, the most

prominent of which were due to several microbial species, for example, *Listeria monocytogenes*, *Escherichia coli*, *Campylobacter jejuni*, *Salmonella* sp., *Shigella* sp., have proven that food safety is under severe threat from food pathogens (Bintsis, 2017; Chlebicz and Śliżewska, 2018; Faour-Klingbeil and Todd, 2020). Unsafe food containing food pathogens, that is, bacteria, viruses, parasites, or fungi, can cause different diseases ranging from diarrhea to cancers (Dwivedi and Jaykus, 2011; Food Safety Education Program, 2016; WHO, 2020).

Centers for Disease Control and Prevention (CDC) estimates that each year 48 million people get sick from foodborne diseases, 128,000 are hospitalized, and 3000 die. Foodborne pathogens cause diseases and deaths in all populations, particularly in groups at risk such as infants, children, elderly, and immunocompromised persons (CDC, 2020; FDA, 2021; WHO, 2020, 2021). The most common microorganisms responsible for the major foodborne illnesses are shown in Table 1.

In addition to the severe health risks caused by foodborne illnesses, they may also threaten international trade and cause significant economic losses. This has been confirmed by the reports received from the USDA's Economic Research Service. These reports indicated that foodborne illnesses cost the United States more than $15.6 billion. *Salmonella* tops the list of foodborne pathogens, with a treatment cost equivalent to $3.6 billion, followed by *Listeria monocytogenes*, which equals $2.8 billion, and then *Escherichia coli* with a value of $271 million (USDA ERS, 2014). This is in addition to the cost of recalls of the products as well (Tyco Integrated Security, 2012).

Hence, there is an urgent need to develop simple, sensitive, specific, robust, reliable, inexpensive, and rapid techniques for food pathogen detection, can perform at the household level and ensure food safety. These requirements comply with new, portable, completely self-administered, on-site DNA test technology called "DNAFoil technology," which does not need expensive equipment or laboratory settings to get the final results in as little as 30 min (El Sheikha, 2019). Additionally, DNAFoil technique has proven to be effective in:

- Detecting food contamination through its ability to detect pork contamination in beef as lower as 0.1% (Meat and Livestock Australia Limited "MLA", 2018);
- Detecting of food adulteration through its ability to detect the adulteration of milk products by vegetal materials as lower as 0.01% (Aronoff *et al*., 2018).

Through five steps (Figure 1), it can be clearly understood as to how this technique works. Sample preparation

Table 1. The most common microorganisms responsible for the major foodborne illnesses.

Foodborne illness or toxin	Associated microorganism	Health risks	Most population group(s) affected	Reference
Listeriosis	*Listeria monocytogenes*	Meningitis, mild illness in pregnant women, in babies (miscarriage, stillbirth, premature birth, potentially fatal infection after birth)	Pregnant women, newborns, the elderly, immuno-compromised individuals	Buchanan *et al*. (2017), Mayo Clinic (2020a)
Salmonellosis	*Salmonella* spp.	Typhoid fever, inflammatory bowel disease, stomach or bowel disorders	All groups	Bintsis (2017), Mayo Clinic (2019)
Shigellosis	*Shigella* spp.	Dehydration, seizures, rectal prolapse, hemolytic uremic syndrome, toxic megacolon, reactive arthritis, bloodstream infections (bacteremia)	Malnourished children, immuno-compromised individuals, the elderly	NCBI (2017), Mayo Clinic (2020b)
Campylobacteriosis	*Campylobacter* spp.	Mild to severe diarrhea, bloody diarrhea, stomach pain, cramps, nausea and/or vomiting, fever, muscle pain	All groups	Bintsis (2017), Ontario Ministry of Health and Long-Term Care (2020)
Botulism	*Clostridium botulinum*	Breathing problems, trouble swallowing, muscle weakness, slurred speech, headache, nausea	All groups	Bintsis (2017), Rasetti-Escargueil (2020)
Toxoplasmosis	*Toxoplasma gondii*	Headache, seizures, lung problems, severe eye infections, e.g., inflammation of retina, enlarged liver and spleen	All groups, especially babies, immuno-compromised individuals	EFSA and ECDC (2016), Mayo Clinic (2020c)
Yersiniosis	*Yersinia* spp.	Fever, abdominal pain, diarrhea (which is often bloody)	All groups, especially children, adults	EFSA and ECDC (2016), Ontario Ministry of Health and Long-Term Care (2018)
Amoebiasis	*Entamoeba histolytica*	Bowel perforation, gastrointestinal bleeding, stricture formation, intussusception, peritonitis, empyema	All groups	NCBI (2016), Park (2015)

without the need to be pre-enriched before analysis is considered the main obstacle in most methods, but the enrichment remains essential for the revival of stressed or injured cells (Cossarizza *et al*., 2017; Valderrama *et al*., 2016). But, through the DNAFoil mechanism, the sample preparation and DNA extraction stages were completed in a single step without the need to use spin-columns and centrifuges. For the amplification stage, cross-contamination is one of the difficulties faced by the commercially available kits used to detect food pathogens, that is, *Salmonella* and *Listeria monocytogenes* (Baraketi *et al*., 2018). In contrast to what happened using the DNAFoil technique, it is obvious that the DNA target amplification is done in one pot master mix without requiring trained staff, using thermos-cycling and cold chain. For the final stage (DNA detection stage), there are several problems generated from DNA electrophoresis and staining such as it being time-consuming, gel preparation, smearing, mutagenicity, toxicity, lower efficiency, etc. (Drabik *et al*., 2016; Hall, 2020). DNAFoil as a detection method during the final stage provides the test strip material, which allows for transport by a capillary force of the target DNA through the detection surface, allowing the target to hybridize specifically to their complementary capture sequences (target DNA fragments are captured in a band). Conjugation of micrometer-sized beads to DNA permits the results to be visualized by the naked eye (visible color reaction), enabling immediate, simple to interpret, cost-efficient, and on-site detection, while eliminating the need for advanced expensive instrumentation (El Sheikha, 2019).

Hence, the aim of this study was to investigate initial validation experiments of the DNAFoil technique to detect food pathogens, that is, *Salmonella* and *Listeria monocytogenes*.

Materials and Methods

Reference materials

Crude bacterial DNA extracts were purchased from the Culture Collection of Switzerland (CCOS), and experiments were conducted at the Swiss Decode labs in Renens, Switzerland. Live strains tested were procured from the Pasteur Institute (France). The strains tested were divided into two groups one for inclusivity and the other for exclusivity toward *Salmonella* and *Listeria monocytogenes* as follows. Firstly, the strains were tested for inclusivity and exclusivity toward *Salmonella*: (i) For inclusivity (*Salmonella Montevideo*, *Salmonella Heidelberg*, *Salmonella mbandaka*, *Salmonella enteritidis*, *Salmonella agona*, *Salmonella Indiana*, *Salmonella infantis*, *Salmonella arizonae* IIIa, *Salmonella senftenberg*, *Salmonella cerro*, *Salmonella Virchow*, *Salmonella anatum*, *Salmonella Newport*, *Salmonella thyphimurium*, *Salmonella arizonae* IIIb, *Salmonella saintpaul*, *Salmonella hadar*, *Salmonella enteritidis abony*); (ii) For exclusivity (*Listeria monocytogenes*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus brasiliensis*, *Candida albicans*). Secondly, the strains tested for inclusivity and exclusivity toward *Listeria monocytogenes*: (i) For inclusivity (*Listeria monocytogenes*); (ii) For exclusivity (*Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus brasiliensis*, *Candida albicans*). All experiments involving living strains were conducted under BSL2 conditions at the independent microbiology lab in Couternon, France.

Sample preparation, DNA extraction, and amplification stages

DNAFoil mechanism is depicted in Figure 1. Steps 1 and 2 show the DNA extraction, lysing, neutralizing, and stabilizing processes of 200 µL of culture using a barrel without the need to use spin-columns and centrifuges. Briefly, bacteria cells are broken by an alkaline solution that contains chaotropic salts. This allows DNA to be released in the solution. The alkaline pH of the solution is not compatible with downstream DNA amplification; therefore, a neutralization/stabilization step is added, and it consists of buffering the pH with a second solution, which also provides monovalent salts that facilitate the DNA amplification reaction. Amplification stage of DNA target is started via step 3; one drop of extracted DNA is transferred to the reaction tube and is incubated in a water bath at 65°C. Then, using the specific primers and enzymes, the DNA targets will amplify and make multiple copies without using thermos-cycling and cold chain.

End-point assays

After 30 min of incubation at 65°C, DNAFoil strips were dipped into the reaction tube (detection step; 4th step). Migration by lateral-flow caused a positive band's appearance due to colloidal gold concentrating on the DNA capture line without using electrophoresis and staining (detection step; 5th step).

Real-time confirmatory assays

For real-time assays, 1 µL of extract was combined with 24 µL of *Salmonella* and *Listeria monocytogenes* reactions mix and incubated at 65°C. Fluorescence (FAM channel) was monitored every 20 s, and the fluorescence signal was plotted over time.

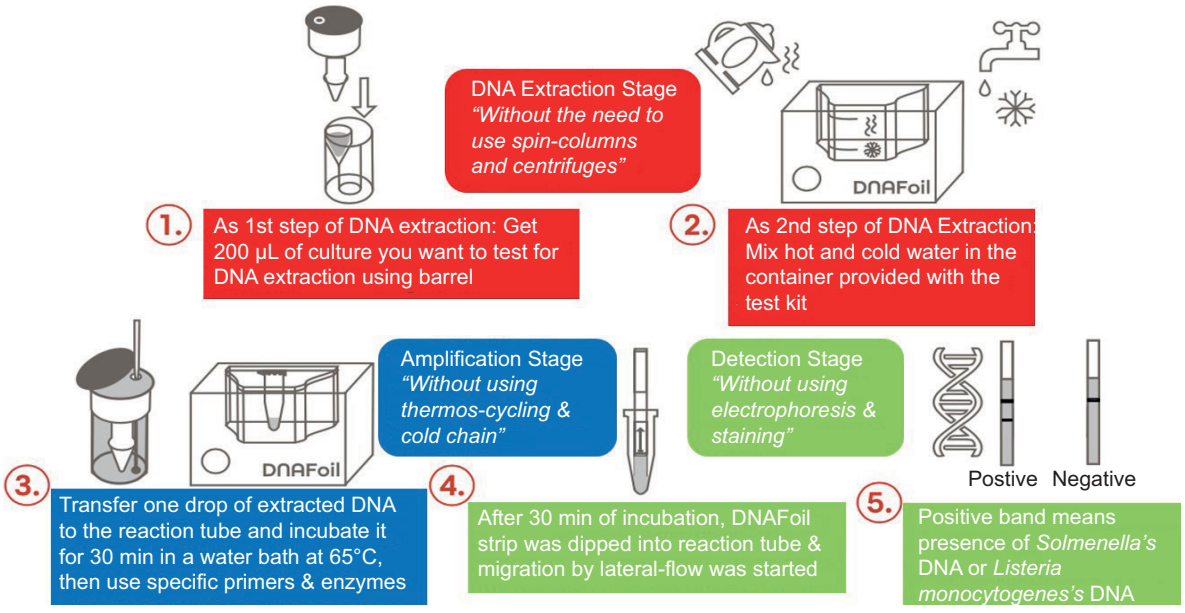

Figure 1. Procedural diagram for the mechanism of food pathogen detection using DNAFoil technology. Source: Adapted from El Sheikha (2019). Reproduced with permission of Elsevier.

Statistical analysis

All data were presented as the mean value ± standard deviation (SD) of independent experiments on various days.

Performance metrics

Accuracy (%)

Results from the experimental specificity (Figure 3A and B) were used to calculate the method accuracy using the following equations:

$$\text{Accuracy (\%) at 10 min} = \frac{(TP+TN)}{(TP+TN+FP+FN)} \times 100 \quad (1)$$

$$\text{Accuracy}(\%) \text{ at 15 min} = \frac{(TP+TN)}{(TP+TN+FP+FN)} \times 100 \quad (2)$$

$$\text{Accuracy}(\%) \text{ at 20 min} = \frac{(TP+TN)}{(TP+TN+FP+FN)} \times 100 \quad (3)$$

where TP, FN, FP, and TN represent the number of true positives, false negatives, false positives, and true negatives, respectively.

Precision (%)

Results from the experimental specificity (Figure 3A and B) were used to calculate the method accuracy using the following equations:

$$\text{Precision}(\%) \text{ at 10 min} = \frac{TP}{(TP+FP)} \times 100 \quad (4)$$

$$\text{Precision}(\%) \text{ at 15 min} = \frac{TP}{(TP+FP)} \times 100 \quad (5)$$

$$\text{Precision}(\%) \text{ at 20 min} = \frac{TP}{(TP+FP)} \times 100 \quad (6)$$

where TP, FN, FP, and TN represent the number of true positives, false negatives, false positives, and true negatives, respectively.

Results

Inclusivity and exclusivity

To confirm the specificity of our method toward *Salmonella*, 18 different *Salmonella* strains, 6 non-*Salmonella* bacteria, and 2 fungi were investigated; also, in the case of *Listeria monocytogenes*, five bacterial strains, two fungi, and *Listeria monocytogenes* were investigated.

Independent microbiology lab prepared cultures containing 10^8 CFU/mL. Swiss Decode analyzed the broth media in a blinded manner. The amplification time for each strain is reported in Figure 2A and 2B.

The standard amplification time used for our DNAFoil kit is 30 min. Samples for which the amplification signal was not detected after 30 min were considered as negative (no presence of *Salmonella* or *Listeria monocytogenes*).

Swiss Decode *Salmonella* solution positively identified the 18 *Salmonella* strains after 10 to 13 min (Figure 2A).

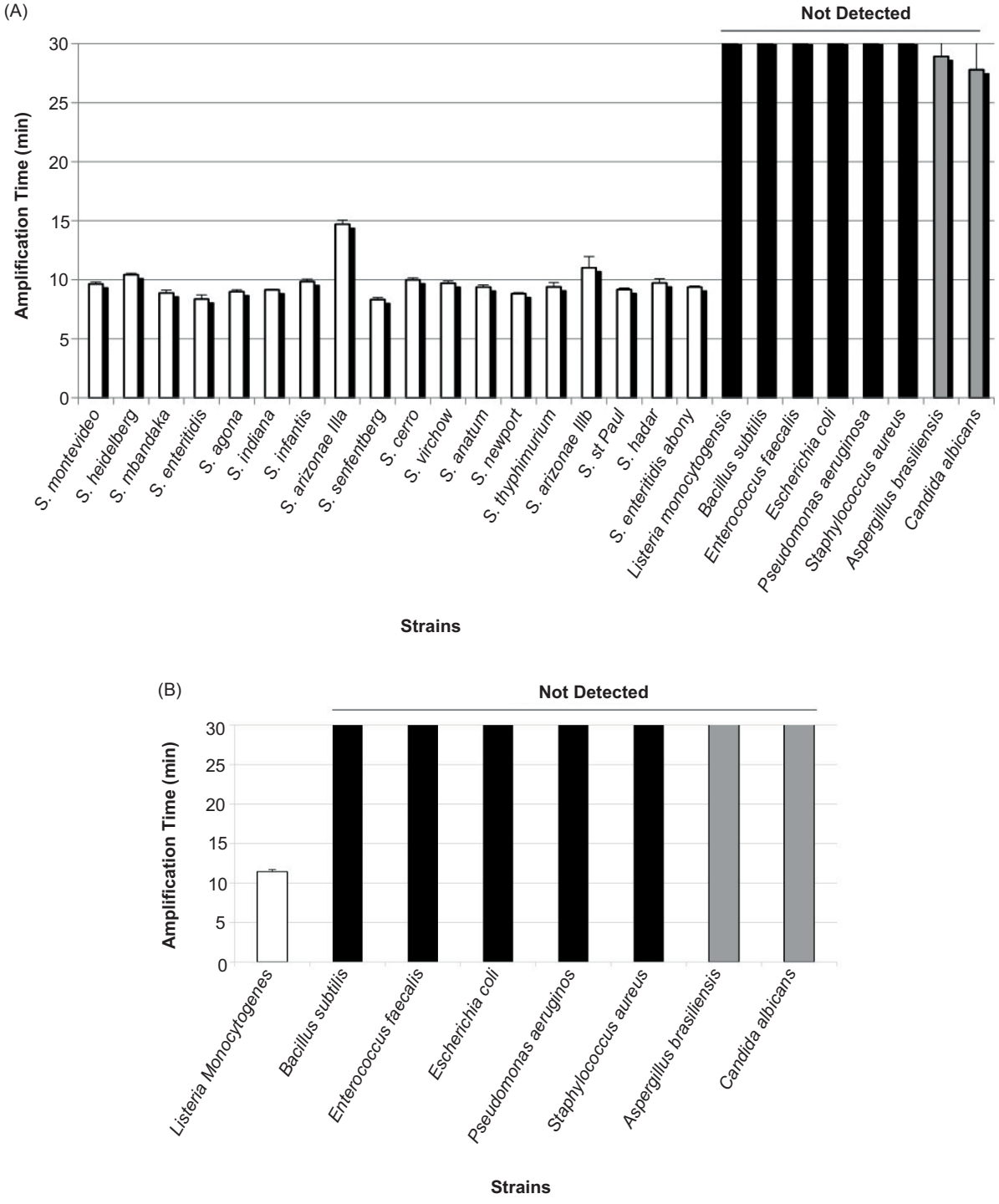

Figure 2. Amplification time—selectivity experiment. (A) Different cultures of *Salmonella* (in white), non-*Salmonella* bacteria (in black), and fungus (in gray) were prepared at an independent microbiology lab. (B) *Listeria monocytogenes* (in white), different bacterial cultures (in black), and fungus (in gray) were prepared at an independent microbiology lab. Broth media (200 µL) was withdrawn from cultures containing 10^8 CFU/mL. Bacteria were lysed according to the standard DNAFoil method. DNA detection was performed with a real-time assay according to the Swiss Decode protocol. Data represent mean ± SD, n = 3.

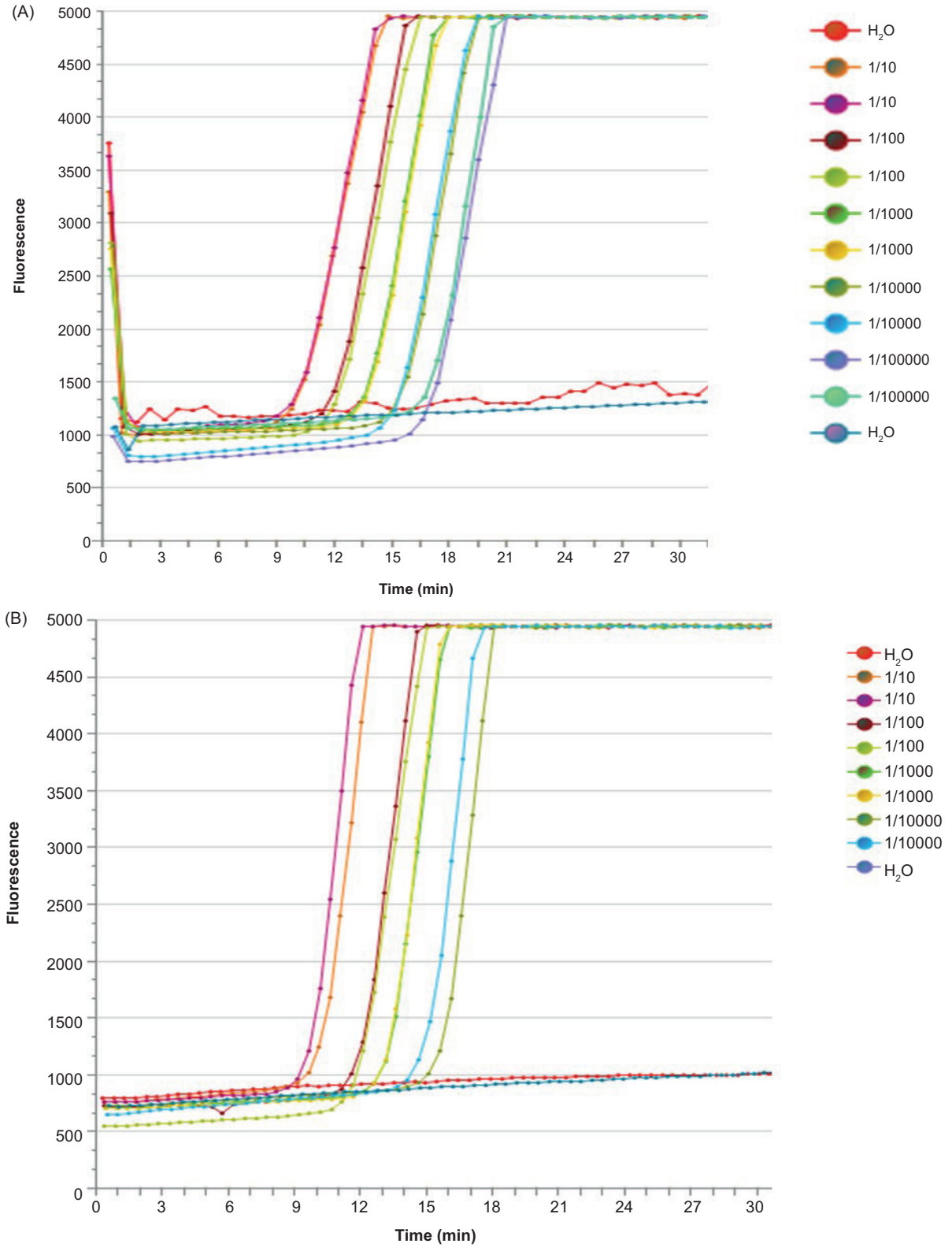

Figure 3. Amplification time-sensitivity experiment. Ten times serial dilution of crude DNA extracts from *Salmonella enterica* and *Listeria monocytogenes* were diluted in 10 mM TRIS pH 8.0 or DNAFoil lysis buffer. One microliter of the lysis solution was taken to run a real-time assay. (A) We can observe that the time needed for the detection of *Salmonella* increases as the amount of DNA present in the sample decreases. However, the detection time is well below the 30 min used as our standard amplification time. (B) We can observe that the time needed for the detection of *Listeria monocytogenes* increases as the amount of DNA present in the sample decreases. However, the detection time is well below the 30 min used as our standard amplification time.

Other non-*Salmonella* strain samples were still negative after 30 min. Our method also allowed *Salmonella*'s identification in broth media samples where *Salmonella* was mixed with several other non-*Salmonella* strains (data not shown).

Regarding the *Listeria monocytogenes*, Swiss Decode *L. monocytogenes* solution positively identified the *Listeria monocytogenes* strains after 11 min (Figure 2B). The other strain samples were still negative after 30 min.

Limit of detection (LOD) with serial dilutions

To determine the sensitivity of our method, a serial dilution of crude bacterial DNA extracts was analyzed. Crude extracts for *Salmonella Enterica* subspc. *enterica* and *Listeria monocytogenes* were obtained from CCOS. Crude extracts containing 10^5 CFU/µL were serially diluted 1:10 either in 10 mM TRIS pH 8.0 or in our DNAFoil lysis buffer. The reactions were analyzed in duplicate by real-time assays (Figure 3A and 3B). The results were similar for both dilution methods (data not shown).

Accuracy and precision (%)

Accuracy and precision (%) are calculated to measure the performance of our method to identify *Salmonella* and *Listeria monocytogenes*. These results are shown in Figure 3A and 3B. The results also showed that the accuracy of our method ranges between 92 and 100%, while the precision value ranged between 88 and 100%.

Point-of-need detection with lateral flow

As real-time thermocyclers may not be present at the point-of-need (i.e., factory), we verified if the DNAFoil *Salmonella* and *Listeria monocytogenes* reactions could be visually detected with the naked eye using a lateral flow strip. Serial dilutions of *Salmonella enterica* and *Listeria monocytogenes* extracts were prepared and amplified as before. After 30 min of amplification at 65°C, the results were confirmed with DNAFoil strips (Figure 4A and 4B).

Discussion

The detection of foodborne pathogens has historically been culture-, or conventional-, or cultural-, or gold-standard-based methods, which were used since the inception of microbiological sampling (Adzitey and Huda, 2010, 2011; Bhunia, 2014). These methods mainly involve enrichment (pre-enrichment and/or selective

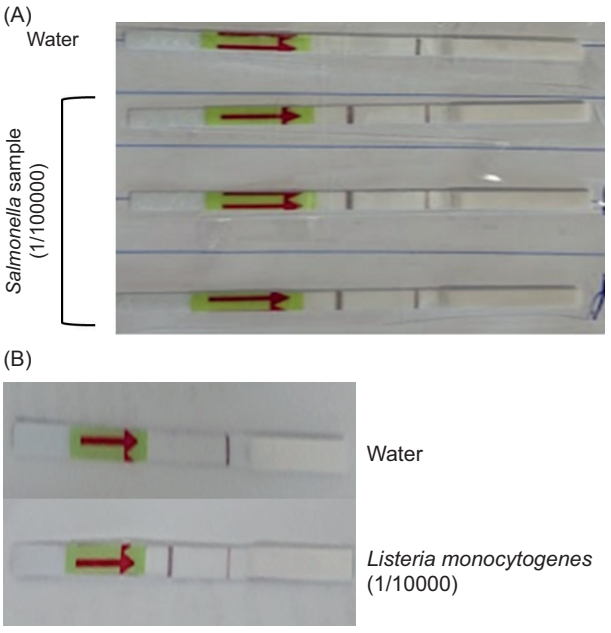

Figure 4. Strips results. (A) Amplified samples from *Salmonella* dilution 1/100,000 were applied on DNAFoil strips (triplicate). A few minutes later, we could see bands appearing. The band on the left confirmed the presence of *Salmonella*, whereas the band on the right is the positive control of the strips. (B) Amplified samples from *Listeria monocytogenes* dilution 1/10,000 were applied on DNAFoil strips. Four minutes later, we could see bands appearing. The band on the left confirmed the presence of *Listeria monocytogenes*, whereas the band on the right is the positive control of the strips.

enrichment) followed by biochemical tests (metabolic fingerprinting), molecular tests (typically PCR [polymerase chain reaction]), or mass spectrometry (Adzitey *et al*., 2011; Corry *et al*., 2003; Ellis *et al*., 2019) to confirm that the isolate is indeed the pathogen of interest. The gold-standard-based methods have the advantages of being inexpensive, detecting only viable pathogens, and yielding isolates that can further be studied (Adzitey and Huda, 2011; Engberg *et al*., 2000). However, they are cumbersome, relatively slow, and less efficient (Foddai and Grant, 2020; Jasson *et al*., 2010; Keramas *et al*., 2004; Li and Zhu, 2017; Myint *et al*., 2006). Regarding the biochemical and mass spectrometry methods, they are rapid, sensitive, and accurate techniques that involve the analysis of entire microbial cells or their extracts (Beale *et al*., 2014; Cevallos-Cevallos *et al*., 2011; Singh *et al*., 2011; Singhal *et al*., 2015; Toscano *et al*., 2018; Wu *et al*., 2016; Yang *et al*., 2015). However, they are labor-intensive, costly, and the reliance on existing spectral databases of the mass fingerprints of known microbes makes mass spectrometry techniques incapable of identifying new species (Anderson *et al*., 2012; El Sheikha and Hu, 2020; Jadhav *et al*., 2018; Mirmajlessi *et al*., 2015; Reta *et al*., 2020). Molecular techniques have the advantage of being

rapid, less laborious, more sensitive, specific, and efficient, compared to the conventional method (Keramas *et al*., 2004; Magistrado *et al*., 2001). Nonetheless, certain components/compounds in foods such as fats, lipids, and salts, enrichment media, or DNA extraction solution can inhibit the sensitivity of PCR-based methods (El Sheikha, 2010; Rossen *et al*., 1992; Wilson, 1997).

To overcome the limitations of traditional methods used for pathogen detection from the side and from the other side to meet industrial and commercial food needs, there is an urgent need for fast, sensitive, accurate, and more efficient detection methods in terms of saving time, labor, and preventing human errors (Baraketi *et al*., 2018; Law *et al*., 2015; Mandal *et al*., 2011; Rajapaksha *et al*., 2019).

Hence, the importance of answering the principal question, namely, why is DNAFoil technology proposed to detect food pathogens? This question is the hypothesis on which the research idea was based, and which the results of this study approved and provided the answers as follows:

DNAFoil is a fast, accurate, precise, sensitive, and reliable technique

As a new technology that needs assessment, the "real-time" amplification technology (real-time PCR) is used to evaluate the efficacy and accuracy of DNAFoil technology (El Sheikha, 2019). Aronoff *et al*. (2018) reported that the efficiency of DNAFoil kit used to detect the vegetal material in milk products (DNAFoil UniPlant) was confirmed using real-time PCR assays. The same authors concluded that the DNAFoil UniPlant kit provides a quick and reliable method to validate product content with less than 1% adulteration of any product, confirming identity and purity in 30 min, without lab equipment, technicians, or scientists. The final report provided by Meat and Livestock Australia Limited (MLA) (2018) illustrated that the DNAFoil kit is able to detect pork contamination in beef as lower as 0.1%.

The present study illustrated that the DNAFoil is a fast-detection technique of *Salmonella* and *Listeria monocytogenes* that can get the final results in as little as 30 min. In addition, the results of this study stated that the Swiss Decode *Salmonella* and *L. monocytogenes* solutions can detect as few as 1 and 10 copies of DNA per microliter, respectively. However, the commercially available kits used to detect food pathogens, that is Salmonella and Listeria monocytogenes, which are based on nucleic acid for detection, are characterized by reliability, high specificity, and sensitivity; they are limited by the difficulties of:

- differentiating the viable cells from nonculturable cells;
- the primers' design.

Moreover, these kits require trained staff to avoid cross-contamination (Baraketi *et al*., 2018). Table 2 evaluates the commercial kits used to detect *Salmonella* and *Listeria monocytogenes*. In terms of performance metrics (accuracy, precision %), the results show promising performance, to be used for *Salmonella* and *Listeria monocytogenes* detection.

The DNAFoil technology is efficient in terms of cost and quality control applicability

In terms of cost. DNAFoil is available to the partners (academic and industry) that accede to the Early Access Program. The fee to enter the Early Access Program is

Table 2. Commercially available kits based on nucleic acid methods for the detection of foodborne pathogens.*

Pathogen	Commercially available kits	Sensitivity	Sample matrix	Company
Salmonella sp.	BAX® System Standard PCR assays for *Salmonella*	10^4 CFU/mL, after enrichment	Poultry, dairy, fruits, vegetables, bakery products, pet food, environmental samples	HYGIENA
	BAX® System Real-time PCR assay for *Salmonella*	10^4 CFU/mL, after enrichment	Meat, poultry, dairy, fruits, vegetables, bakery products, pet food, environmental samples	HYGIENA
	GeneQuence® for *Salmonella*	1–5 CFU/25 g	Food and environmental samples	HYGIENA
Listeria monocytogenes	BAX® System PCR Assay for *L. monocytogenes*	10^5 CFU/mL, after enrichment	Variety of food types	HYGIENA
	BAX® System PCR Assay for *L. monocytogenes* 24E	10^4 CFU/mL, after enrichment	Dairy, meat, fish, vegetables, environmentals	HYGIENA
	BAX® System Real-Time PCR Assay for *L. monocytogenes*	10^4 CFU/mL, after enrichment	Dairy, ready-to-eat meat, seafood, vegetables, environmental samples	HYGIENA
	GeneQuence® for *L. monocytogenes*	1–5 CFU/26 g	Food and environmental samples	NEOGEN

*Source: Baraketi *et al*. (2018). Licensed under Creative Commons Attribution 3.0. PCR, polymerase chain reaction.

€ 990 and the program makes access free of charge for a kit of 5 tests. The final cost per test is negotiable, and it is usually much cheaper than the traditional detection methods (Aronoff *et al*., 2018; Lüdin *et al*., 2018).

In terms of quality control applicability. The DNAFoil output gives a (±) answer, for example, while using DNAFoil Pork Test Kit, a positive result indicates the presence of porcine DNA in the sample tested. This is enough for an inspector to take instant action. The strips with the results can be stored as evidence to prove that adequate controls are in place during manufacturing. In the case of litigations, the Swiss Federal Lab concluded that DNA can be easily extracted from the strip, and such DNA can be used for more lab tests (Lüdin e*t al*., 2018).

Conclusions, Remarks, and Future Trends

In this study, the developed DNAFoil *Salmonella* and *Listeria monocytogenes* reactions correctly identify a wide range of *Salmonella* strains and also *Listeria monocytogenes*, among other bacterial strains. These reactions are specific and sensitive, with a virtual limit of 1 and 10 CFU detection per reaction for *Salmonella* and *Listeria monocytogenes*, respectively. In terms of accuracy and precision, the results show promising performance in which the accuracy ranged between 92 and 100% and the precision ranged between 88 and 100%. Real-time PCR may not be present at the point-of-need (i.e., factory); therefore, the DNAFoil *Salmonella* and *Listeria monocytogenes* reactions could be visually detected with the naked eye using a lateral flow strip. The strips with the results can be stored for quality control in place during manufacturing and also can be used for more lab tests. The results of the present study revealed and confirmed several advantages that considered DNAFoil fact sheet as follows: the DNAFoil is easy to use and does not require specialized skills to perform testing; the DNAFoil is standalone and does not require laboratory equipment other than a source of boiling water. In the absence of boiling water, the kit is compatible with a standard thermoblock; the DNAFoil detects specifically each target without any cross-reaction; the final results can be interpreted with the naked eye with a lateral flow dipstick without the need for thermocyclers, electrophoresis, and staining.

Food pathogen detection approaches have become ever more substantial for all of the food chain components, that is, consumers, producers, and legislators (El Sheikha, 2015; Hoffmann and Scallan, 2017). The embodiments of food pathogen risks in food products continue to evolve in different forms that have resulted in tremendous improvements in analytical methodologies to detect the food pathogens (Hemalata and Virupakshaiah, 2016). Although DNA-based techniques have proven to be the best detection tools in food pathogen detection, at the industrial level its practical application has to go a long way (El Sheikha *et al*., 2018; Zhao *et al*., 2014). Hence, the demand for a novel, rapid, easy, potent, and universal technology for food pathogen detection is still an urgent need. Therefore, it is hoped that the DNAFoil technology could be a powerful tool that meets all of the requirements for food pathogen detection and its applications either at the household or industrial level.

As a future trend, more applications are recommended for DNAFoil technology as a food pathogen detection tool on different food matrices. Moreover, the DNAFoil test kits for *Salmonella* and *Listeria monocytogenes* are qualified for further validation using ISO16140.

Compliance with Ethical Standards

Acknowledgments

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Conflict of Interest

The author declares that there is no conflict of interest.

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