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Chemiluminescent optical fibre genosensor for pork meat detection.

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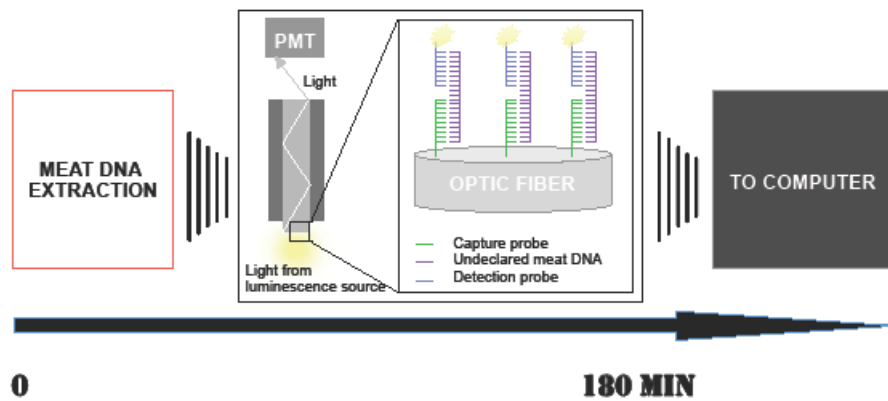
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GRAPHICAL ABSTRACT



Highlights pork meat detection

- pork meat detection using specific DNA probes targeting *Sus Scrofa* mitochondrion genome
- the time required for the detection of samples was of about 2.5 h including DNA extraction
- high sensitivity has been obtained by the genosensor able to detect 1% pork meat in mixtures

Abstract

An easy, rapid and sensitive method for the detection of meat species is important to identify adulteration or fraudulent substitution in raw meats with undeclared species. We report herein the development of an optical fibre genosensor able to detect low level of pork (*Sus Scrofa*) meat in mixtures of minced meat obtained by an optimized and faster DNA extraction protocol. Our approach uses highly specific and sensitive DNA capture and secondary probes designed out of the mitochondrion genome of *Sus Scrofa*. Once DNA capture probes were immobilized on optical fibres, we demonstrate their putative application as genosensors in detecting *Sus Scrofa* DNA in purified samples obtained first using a commercial DNA extraction kit and thereafter using our improved extraction method. Furthermore, analysis of beef (*Bos taurus*) minced meat samples also show that the assay can determine 1% (w/w) of *Sus Scrofa* DNA. The sensitivity obtained by the optical fibre and the short time required for the results (about 2.5 h) indicate the usefulness of the system. We envision that this genosensor may serve as a diagnostic tool for food sample screening using a technology that is accurate, simple to perform and has the potential to be used in portable format.

Keywords: DNA extraction, pork meat, optical fibre genosensor, DNA specific probes

1. Introduction

The demand for meat has increased rapidly in recent decades, from 71 million tons in 1961 to 284 million tons in 2007, and it increased by 20% in just the last 10 years. Pork is the most widely consumed meat in the world, followed by poultry, beef, and mutton [1].

In an investigation performed on 100 meat products, meat from undeclared species was found in 22 % of cases, primarily with poultry substituting beef [2]. Moreover, the European horse meat scandal of 2013 highlighted the need to detect the addition of horse meat to beef, but also the need for

detecting the adulteration of any meat with undeclared species. Adulteration of meat products is a continuing problem for economic, ethical, moral, and religious reasons. Muslim and Jewish consumers are prohibited from consuming pork, as well as there is a potential danger of serious allergic responses or intolerance to proteins derived from specific animals [3].

The test method should allow the identification of the meat species, both raw and processed to verify conformity between the food labelling and the effective composition in order to ensure food control and consumer protection. Many methods based on proteomics and genomics, such as electrophoretic techniques, including capillary electrophoresis methods, enzyme-linked immunosorbent assays (ELISA), PCR, and mass spectrometry have been developed for the identification of species [4]. Electrophoretic techniques results are difficult to interpret when mixtures of more than one species are involved, they are time consuming and only semiquantitative [5]. Analytical techniques, such as chromatography and mass spectrometry methods with focus on protein profiles, are proposed and include qualitative detection of meat from a variety of animal species [6, 7, 8] but even if accurate they are expensive, laborious and not suitable for on site analysis and rapid response.

DNA-based methods have proved to be reliable and robust tools for meat speciation, but suffer from time-consuming extraction methods that require several steps of filtration and centrifugation, and are often based on expensive commercial kits [9, 10, 11, 12, 3]. Therefore, new, rapid and sensitive authentication methods require development to ensure the easy identification of undeclared species. In order to identify unexpected meats, specific biosensing techniques with high sensitivity and low costs are of increasing importance for food control of the raw materials. In this perspective, optical fibre sensors are ideal transducers governed by the Snell's law, having several advantages that make them attractive options for use in sensing applications [13]. Since recently, optical fibre biosensors based on chemically generated luminescence have been shown to detect analytes in very low quantity with high specificity and sensitivity [14, 15]. We propose herein a new, rapid, portable and highly sensitive fibre optic genosensor for the identification of 1% of pork meat in mixtures for

meat authenticity evaluation. A couple of new specific and sensitive DNA probes targeting the mitochondrion genome of pork (*Sus scrofa*) were designed by us and utilized in our method which also employs a simple and fast DNA extraction protocol based on proteinase K and lysis buffer treatment. The fast lysis allowed to analyze samples in few hours while still maintaining high sensitivity.

2. Material and Methods

2.1 Reagents

Oligonucleotides were purchased from Integrated DNA Technologies Pte Ltd (Singapore) and were resuspended in sterile MilliQ water to give stock solutions of 100 µM. Emsure® Iso hydrogen peroxyde 30% (Perhydrol® for analysis) and Emsure® hydrochloric acid fuming 37% were purchased from Millipore, USA. Clarity™ Western ECL Substrate peroxidase solution, luminol/enhancer solution and EDTA were from Biorad, USA. Wizard® Genomic DNA purification kit was purchased from Promega, USA. All other chemicals, including Proteinase K (PK), Triton X-100, Guanidine Hydrochloride, SDS, SSC 20X, NaCl, and positively charged nylon membrane were purchased from Sigma-Aldrich Pte Ltd (Singapore) unless otherwise specified. Lysing buffer was prepared using Gu-HCl 3M, EDTA 1 mM pH 8.0, NaCl 100 mM, triton-X100 (2%), SDS (1%).

2.2 Meat Samples

Chicken, beef, lamb, turkey, and minced pork and beef samples were purchased from local supermarkets. 10 g pork (P100%), 10 g beef (B100%) and samples of 10 g mixtures of pork and beef (P10%-B90%; P5%-B95%; P1%-B99%) were prepared and used for analyses.

2.3 DNA extraction

DNA was extracted from 20 mg tissue samples of chicken, beef, lamb, turkey, pork and beef using the Wizard® Genomic DNA purification kit with the addition of proteinase K (17.5 µL at 20

mg/mL), incubated for 3 h at 55°C, and finally quantified using the NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, USA).

Pork P100%, beef B100%, and the mixtures P10%-B90%, P5%-B95% and P1%-B99% were also used for DNA extraction. 10 g of previously described meat samples were added to 40 mL of physiological solution (0.9% NaCl) in a Stomacher® bag and homogenized for 3 min. 10 µL of PK (20 mg/mL) were added to 1 mL of the homogenized liquid from the Stomacher® bag and the solution was incubated for 30 min at 37 °C in a 2 mL tube: after incubation the samples were immediately placed on ice. Alternatively, 1 mL of the lysis buffer and 20 µL of PK (20 mg/mL) were added to 1 mL of the homogenized liquid from the Stomacher® bag: the samples were incubated for 30 min at 37 °C in a 2 mL tube: after incubation the samples were immediately placed on ice. This protocol took about 40 minutes.

2.4 Design of DNA probes

Capture and detection probe sequences were built using the rules of no strong homo dimers, no strong hetero dimers, no strong hairpin formation, similar melting temperature and were checked using the online tools of IDT's Oligo Analyzer v.3.1. Detection and capture probes sequences were analysed using BLASTN 2.3.1. The selected target was a portion of the mitochondrion genome, accession number: KT372134.1, KM520149.1, KJ46666.1 and AF034253.1. The sequence of the dig1 probe (capture probe, 28 mer) was 5'-DIG- TAAACCCCGATAGACCTTACCAACCCTT-DIG-3', and the sequence of the dig2 probe (detection probe, 26 mer) was 5'-DIG-AAGCACAATCATAGCACATAAAAACG-DIG-3'. Both probes were labelled with digoxigenin at both ends. To evaluate the sensitivity of the probes, the following DNA sequences complementary to the dig probes were synthesized and used as a template. Compl. dig1: 5'- AAG GGT TGG TAA GGT CTA TCG GGG TTT A -3' (28 mer) and Compl. dig2: 5'- CGT TTT TAT GTG CTA TGA TTG TGC TT -3' (26 mer).

2.5 Dot blot assay

The following samples were used for the dot blot assay: (a) 1 μ L of the oligonucleotide complementary to the capture dig1 probe (Compl. dig1) and 1 μ L of the oligonucleotide complementary to the detection dig2 probe (Compl. dig2) at the concentrations of 100 ng/ μ L, 10 ng/ μ L, 1 ng/ μ L, 0.1 ng/ μ L, 0.01 ng/ μ L, 1 pg/ μ L, 0.1 pg/ μ L, 0.01 pg/ μ L, 1 fg/ μ L were spotted onto the positively charged nylon membrane to evaluate the sensitivity; (b) 1 μ L of DNA from pork, chicken, beef, lamb and turkey extracted with the Wizard[®] Genomic DNA purification kit were spotted at concentrations of 100 ng/ μ L onto the positively charged nylon membrane to test specificity of the probes; (c) 1 μ L of DNA from pork P100%, beef B100%, and mixtures of pork and beef (P10%-B90%, P5%-B95% and P1%-B99%) extracted with PK or with lysing buffer added with PK (LPK) were spotted onto the positively charged nylon membrane. The capture probes were used at a final concentration of 100 ng/mL in the hybridization buffer. A positive control consisting in 1 μ L of the sequence complementary to the capture dig1 probe or complementary to the detection dig2 probe were used in each membrane. Tests were done three times each.

All dsDNA samples were denatured at 95 °C for 10 min and chilled immediately on ice before 1 μ L was spotted on the nylon membranes and cross-linked to the air-dried membranes by exposure to UV light for 10 min.

The membranes were prehybridised in a pre-warmed Dig Easy Hyb (Roche Diagnostic, Mannheim, Germany) buffer for 30 min at the optimal temperature calculated of 44 °C on a shaker. Hybridisation was carried out at 44 °C overnight in the same buffer (Dig Easy Hyb) supplemented with the digoxigenin-labelled capture dig1 probe or digoxigenin-labelled detection dig2 probe after 10 min denaturation at 68°C. At the end of the incubation, the membranes were washed twice with SSC 2X with 0.1% SDS for 5 min at room temperature on a shaker and twice with 0.1 X SSC with 0.1% SDS at the same conditions.

The membranes were then washed twice with a 1X washing buffer (Roche, Singapore) for 5 min on

a shaker. The membranes were incubated in a blocking solution (maleic acid buffer 1X and 10X blocking solution, Roche) for 30 min with shaking. Subsequently, antibody solution (antidigoxygenin-AP diluted in blocking solution 1:5000, Roche) was added and the membranes were incubated for 30 min on a shaker. The membranes were washed twice with washing buffer 1X on a shaker for 15 min. Detection buffer 1X was used to neutralise the membranes that were then incubated with a colour solution (NBT/BCIP, Roche) in the dark without shaking. After 1 hour the reaction was stopped by rinsing the membranes for 5 min in sterile water: finally the results were recorded.

2.6 Preparation of the optical fibre tips

SFS400/440B Superguide G UV-Vis silica fibres (Fiberguide Industries, Stirling, USA) were used for all experiments. The fibres had an original numerical aperture (NA) of 0.22, a core diameter of 400 μm (refractive index of 1.457 at 633 nm) and a surrounding silica cladding with a width of 40 μm (refractive index of 1.44 at 633 nm), in addition to a 150- μm -thick silicon buffer and a 210- μm -thick black Tefzel[®] jacket. The length of a single fibre used in the experiments was 25 cm. The black Tefzel[®] jacket and silicon buffer were mechanically stripped away using a fibre stripping tool (Micro-Strip[®], from Micro-Electronics Inc., USA) to expose a 2 mm naked optical fibre core tip.

In order to prepare the fibres for the silanization procedure, they were soaked in a 1:1 methanol/37% (v/v) HCl solution for 20 min to purify the newly exposed fibre core from micro-contaminants. After sonication for 20 minutes, the fibre tips were acid-cleaned with Piranha solution (30% H_2O_2 : 96% H_2SO_4 in the ratio 3:7) for 10 min at 90 °C to enhance the exposure of the hydroxyl groups on the silica surface.

2.7 Silanization of the optical fibre

The silanisation procedure was performed as described by [16]. After a washing step with deionized water, the glass tips were dried under N_2 and immersed in 2 mL of 3-

glycidyloxypropyltrimethoxysilane at 85 °C for 1 hour and 30 minutes. After the silanization, the fibres were quickly rinsed twice in sterile water, treated with 11.6 mM of hydrochloric acid 37% at 50 °C for 1 hour, quickly rinsed in sterile water, incubated in a solution 100 mM of sodium periodate in 10% (v/v) acetic acid for 1 hour in the dark at room temperature, and again rinsed with sterile water.

Fibres were incubated in 100 µL of DNA capture probe solution (50 ng/µL) overnight at 4 °C. The unreacted aldehydes were exposed to a blocking solution 3% albumin from bovine serum in 0.1% (v/v) Tween 20 Tris buffer solution (TBS: sodium chloride 8 g/L, potassium chloride 8 g/L, Tris base 3 g/L, pH 7.5) at 37 °C for 2 h. The C,N-dihydro-addition reaction was used to stabilise the Schiff base via the oxo-dialkylimino-bisubstitution mechanism, in which the fibres with unsaturated secondary amines were dipped into a 0.3 M sodium cyanoborohydride in water solution for 40 min at room temperature. The fibre tips were washed three times in 3% (w/v) BSA – 0.1% (v/v) Tween 20 TBS for 5 min each.

2.8 DNA hybridization

The DNA-modified fibres were dipped in the hybridisation buffer (3% (w/v) BSA – TBS) containing DNA extract from pork meat samples using Wizard® Genomic Purification kit (as positive samples), DNA extracted by PK or LPK from meat mixtures of pork meat at various percentages, DNA extracted from beef meat (negative sample) and a biotin-labelled secondary probe (60 ng/µL). The DNA and detection probe were added together in one step, and the mixture was incubated for 1 h at 44 °C. Prior to hybridisation, DNA and detection probes were incubated at 90 °C for 5 min and immediately chilled on ice to ensure that the DNA sequences did not form secondary structures.

2.9 Chemiluminescence detection

The fibre tip surfaces were washed three times in 0.05% (v/v) Tween 20 TBS for 5 min each and

incubated in a diluted solution of avidin-peroxidase in TBS (1.2 μ L in 2 mL) for 1 hour at room temperature in the dark. The fibres were washed three times in 0.05% Tween 20 TBS. Chemiluminescence measurements were conducted using a Hamamatsu HC135-01 Photo Multiplier Tube (PMT) Sensor Module, combining the sensitivity of a photomultiplier tube with the intelligence of a microcontroller. The instrument was placed in a light-tight box equipped with a manual shutter (71430, Oriel) in front of the detector, and a custom-made lever outside the box was used to move the slide shutter of the photon counting unit inside. The far end of each fibre was held by a fibre holder (FPH-DJ, Newport) and placed into an adjustable single-fibre mount (77837, Oriel). The modified optical fibre tips were dipped in 240 μ L of peroxidase solution and luminol/enhancer solution combined in a 1:1 (v/v) ratio. Each measurement was taken as the mean value of the photon counts for a period of 50 s, and each measurement point is presented as the mean and standard deviation of a single fibre set (5 fibres) reported in relative light units (RLU).

3. Results

3.1 Sensitivity and specificity of the probes by dot blot tests

The sensitivity of the digoxigenin-labelled probes at the concentration of 10 pmol/mL was of 1pg/ μ L for both the capture dig1 probe and the detection dig2 probe. The positive spots obtained for the two dig labelled probes were at concentrations ranging from 100 ng/ μ L to 1 pg/ μ L and the positive spots obtained for the detection dig2 probe using the same concentrations for the complementary sequence are shown in Fig.1.

The specificity of the digoxigenin-labelled probes was evaluated by spotting on the nylon membrane 1 μ L (100 ng/ μ L) of the DNA solution extracted using the Wizard[®] Genomic DNA purification kit. Both the capture dig1 and the detection dig2 probes were specific for pork as only the DNA spots of pork showed the expected blue colour. All other tested meat samples (chicken, beef, lamb, and turkey) resulted negative using the conditions reported in the "*dot blot assay*" section as shown in Fig. 2 and Fig. 3. The negative spots obtained for chicken, beef, lamb, and

turkey at 100 ng/μL (the highest concentration used in the tests) demonstrated the specificity of the two new designed probes dig1 and dig2 for pork meat. Indeed, pork samples showed positive results also when only 0.1 ng/μL were used.

3.2 Dot blot for sample detection

The method proposed to extract DNA from mixtures of meat samples is rapid as the time required to obtain the DNA to use in further tests takes 40 min.

The DNA extracted from pork, beef and mixtures of pork and beef using the PK treatment gave positivity for all samples P100%, P10%-B90%, P5%-B95% and P1%-B99 tested. The extracts obtained with lysing buffer and PK gave positivity only for samples P100%, P10%-B90% and P5%-B95%. The correspondence between the percentages of pork meat added to the Stomacher bags and the amounts of the meat used for the extraction of the DNA are reported in Table 1. Starting from a 10 g sample mixture where 1% was pork meat (2 mg), we obtained a positive response from the biosensor when the lysing enzyme was used during the extraction. As reported by Cai et al. [12] it is possible to correlate the amount of pork meat used for the extraction and the amount of DNA obtained. Based on their formula $y = 18.10x + 6.968$ [12] the positivity obtained in this work with 2 mg of pork meat in the sample P1%-B99% could correspond to 43.16 ng of DNA in the 1 mL used for extraction. It means that the amount of pork DNA obtained from the sample P1%-B99% that was positive in the dot blot was about 43 pg as 1 μL was spotted. The sensitivity obtained for the two probes at 1 pg/μL supported the results. The positivity obtained by the fibre optic when using the DNA extracted from the P1%-B99% corresponded to a sensitivity of the test of 8.6 pg/ μL as 20 μL of the extracts were used for the analyses (Table 2). This is the detection limit of our sensor as it gave a positive response. The DNA extraction method used for these samples took 30 min after the addition of the lysing buffer and proteinase K to the meat homogenates, reducing the time required to get the DNA of about 5 h compared to the kit method.

3.3 Chemiluminescent optical fibre measurements

The results obtained using as target the DNA extracted with Wizard[®] Genomic Purification kit from pork meat samples at the different concentrations (1 ng/ μ L, 2.5 ng/ μ L, 5 ng/ μ L, and 7.5 ng/ μ L) are shown in Figure 4. All experiments were carried out using negative controls including DNA from beef meat sample (5 ng/ μ L), no addition of DNA, no addition of the amino-capture probe, no addition of DNA, no addition of the amino-capture probe and no use of the avidin-HRP conjugate.

RLU values ranged from 132 RLU detected for the negative "no addition of the amino-capture probe" to the higher value detected for the 7.5 ng/ μ L of DNA from pure pork that was 330 RLU. A proportional increase in RLU values was obtained, in fact from the lower concentration of pork (1 ng/ μ L) with 218 RLU, 281 RLU were obtained for 2.5 ng/ μ L, 281 RLU for the 5 ng/ μ L and 330 RLU for the last concentration tested at 7.5 ng/ μ L of DNA from pure pork meat.

The signal obtained for the negative samples (a) without capture probe and DNA, (b) with capture probe and without DNA, was always below 160 RLU. From Figure 5 it is possible to note that the presence of a higher amount of pork in the mixed samples analysed produces increased RLU values, from 160 RLU without the target, to 249 RLU with 1% pork meat, to 393 RLU when 10% pork meat is present.

The samples extracted using proteinase K resulted negative when the fibre optic genosensor was used. Conversely samples obtained by the mixtures of beef and pork meat at various percentages, extracted using the lysing buffer with the addition of proteinase K, tested positive for P10%-B90%, P5%-B95% and P1%-B99% showing the RLU values of 393 (SD 74), 277 (SD 26) and 249 (SD 19) respectively, as reported in Figure 5. The detection of the samples has been obtained in 2.5 h including the capture probe hybridization step.

4. Discussion

The meat speciation commercial kits usually utilize 20 mg of tissue, but these small amount of sample cannot be an homogeneous mixture when different meat species are used. For this reason commercially available kits may not provide a representative sample for food analyses.

In this work 10 g of meat were considered as representative sample and were used through our study. The dig labelled probes were shown to hybridize on the nylon membrane with 1 μ L of the meat extract obtained after the meat treatment with proteinase K or lysis buffer containing proteinase K. This result showed that the quality of the extracted DNA was not affected by the extraction methods used. This is consistent with previous studies that demonstrated the use of the DNA extracted by proteinase K as template in PCR protocols [17]. The addition of a lysis buffer increased the success of the detection as it improved the disruption of the cells and allowed the DNA to hybridize to the probes both during the dot blot and the fibre optic measurements. The new probes were specific permitting their utilization in mixtures of meat down to 1% of pork in beef. Indeed, no positivity was obtained for 100% beef both by dot blot or fibre optic tests confirming the specificity already obtained using the DNA extracted with the Wizard[®] Genomic DNA kit in the dot blot assay and in the fibre optic measurement. The different results obtained in terms of sensitivity for the samples extracted by proteinase K and the samples extracted using the lysis buffer with the addition of proteinase K, can be justified looking at the different amounts of extracts used for these purposes, and at the sensitivity of the method. The negative result obtained for the P1%-B99% in the dot blot can be explained by the small volume used in the test, 1 μ L, whereas the positivity obtained with the fibre optic probe is due to the use of a 20 μ L sample size. Moreover, the detection by chemiluminescence increases the sensitivity compared to the dot blot method that is based on colorimetry. Negative results were obtained with the fibre optic genosensor when when the used extracts were obtained just using proteinase K. This can be explained by the presence in the solution of an high content of cell debries and particles, that may interfere with the hybridization between capture and detection probes. Instead, in the dot blot assay only one probe is requested for

the hybridization step that produces the positive colored spots.

The method is sensitive as it gives positive results from small amounts of sample used for the DNA extraction (1 mL from the homogenization bag), although we need to point out that we started from a weight of 10 g of meat. One should note that the processed meat particles are rarely smaller than 20 mg in size. According to the paper of Cai et al. [12] the applied formula can only be indicative of the quantity of the extracted DNA: this formula takes not into consideration certain conditions such as uniformity of the sample, errors of the operators, and the method used for the extraction of the DNA which can all affect the results. Nevertheless, the proposed protocol is very sensitive as demonstrated by the data reported in the paper. The amount of obtained RLU increases with the increase of the pork meat percentage in the mixture (1%P-99%B, 5%P-95%B and 10%P-90%B): our results showed that the genosensor clearly detected small percentages of target (starting from 8.6 pg of pork meat) also in the presence of a large amount of non target species (1:100 ratio). Our data show that the proposed fibre optic genosensor is specific, sensitive, simple and fast as it enables to obtain the results in about 2.5 hours including the fast DNA extraction step, that does not require complex extraction methods or expensive extraction devices extraction.

5. Conclusions

During last decade meat fraud, the mixture of low quality meat or cheaper meat with high quality meat, has been found all over the world last decade. To protect consumer and industry it is important to be able to detect fraud in a short time and with high sensitivity. Based on these considerations our method would be helpful in pork meat detection, as it is fast, simple, cheap and allows the processing of many samples within a short time. The developed protocol ensure the extraction of DNA of good quality, as it can be used directly in the biosensor test, in 40 min, allowing the reduction of the time required for the obtainement of the results to 2.5 h. This means that authorities can take a decision and/or measure in a day. Moreover, this test doesn't require high technology steps for the preparation of the sample. A future step can lead to the implementation of

the system in a complete portable device which could be helpful to the food monitoring authorities to protect consumers from fraude operating in the field, avoiding the transportation of the samples to a laboratory.

Future perspectives:

The authors have proposed a genosensor that lends itself to the possibility of being useful for authorities involved in controls.

Author contributions

All authors have read and approved the final manuscript. All authors contributed to the manuscript realization.

Declaration of conflicting interests

The authors claim that there are no conflicting interests either from the funding source or their institutional affiliations. We thus state that there are no conflict of interest from the authors.

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Figure Captions

Figure 1. Dot blot spots of capture dig1 probe (row a) and detection dig2 probe (row b) using their complementary sequences as a target. Spots of 1 μL of the sequences complementary to the probes at concentrations of 100 ng/ μL (1), 10 ng/ μL (2), 1 ng/ μL (3), 0.1 ng/ μL (4), 0.01 ng/ μL (5), 1 pg/ μL (6). Dig1 and dig2 probes have a 100 ng/mL final concentration in hybridization buffer.

Figure 2. Dot blot spots of capture dig1 probe (100 ng/mL final concentration in hybridization buffer). DNA from pork, chicken, beef, lamb and turkey were extracted with the Wizard(R) Genomic DNA purification kit. 1 μL spots of DNA from pork 100 ng/ μL (1) and 0.1 ng/ μL (2), chicken 100 ng/ μL (C), beef 100 ng/ μL (B), lamb 100 ng/ μL (L) and turkey 100 ng/ μL (T).

Figure 3. Dot blot spots of detection dig2 probe (100 ng/mL final concentration in hybridization buffer). DNA from pork, chicken, beef, lamb and turkey were extracted with the Wizard(R) Genomic DNA purification kit. 1 μL spots of DNA from pork 100 ng/ μL (1) and 0.1 ng/ μL (2), chicken 100 ng/ μL (C), beef 100 ng/ μL (B), lamb 100 ng/ μL (L) and turkey 100 ng/ μL (T)

Figure 4. Validation of the optical fibre system with the DNA extracted from pork and beef meat using Wizard® Genomic DNA purification kit. In all the histograms the light intensities are expressed in relative light units (RLU) emitted by the samples. Each measurement is presented as the mean of 5 fibers (n=5), the bars correspond to the standard deviation (SD) value. Samples description: a) without capture probe and DNA, 158 RLU and SD 13; b) without capture probe and with DNA, 132 RLU and SD 28; c) with capture probe and without DNA, 157 RLU and SD 8; d) DNA extracted from beef meat at 5 ng/ μL , 166 RLU and SD 20; e) DNA extracted from pork meat at 1 ng/ μL , 218 RLU and SD 26; f) DNA extracted from pork meat at 2.5 ng/ μL , 281 RLU and SD

22; g) DNA extracted from pork meat at 5 ng/ μ L, 311 RLU and SD 26; h) DNA extracted from pork meat at 7.5 ng/ μ L, 330 RLU and SD 11".

Figure 5. Validation of the optical fibre system with the DNA extracted from meat real samples using proteinase K and lysis buffer. In all the histograms the light intensities are expressed in relative light units (RLU) emitted by samples. Each measurement is presented as the mean of 5 fibers (n=5), the bars correspond to the standard deviation (SD) value. Samples description: pork (P) and beef (B). a) without capture probe and DNA, RLU 160 (SD 13); b) with capture probe and without DNA RLU 160 (SD 8); c) DNA extracted from 1%P-99%B meat mixture RLU 249 (SD 19); d) DNA extracted from 5%P-95%B meat mixture RLU 277 (SD 26); e) and DNA extracted from 10%P-90%B meat mixture RLU 393 (SD 74).

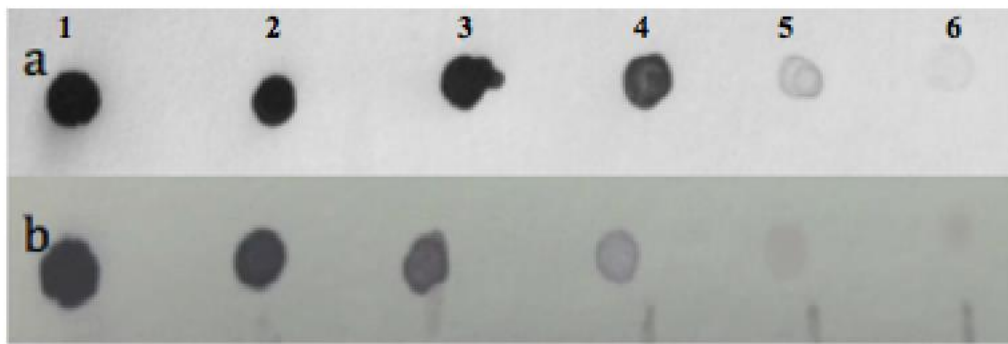


Figure 1.

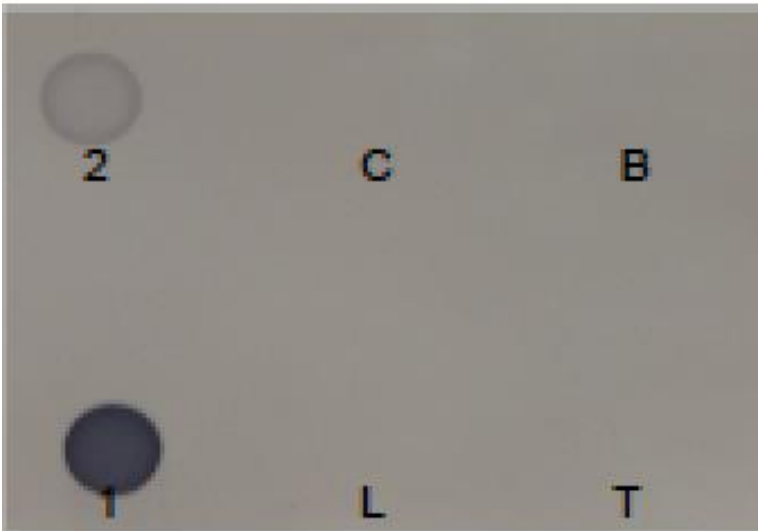


Figure 2.

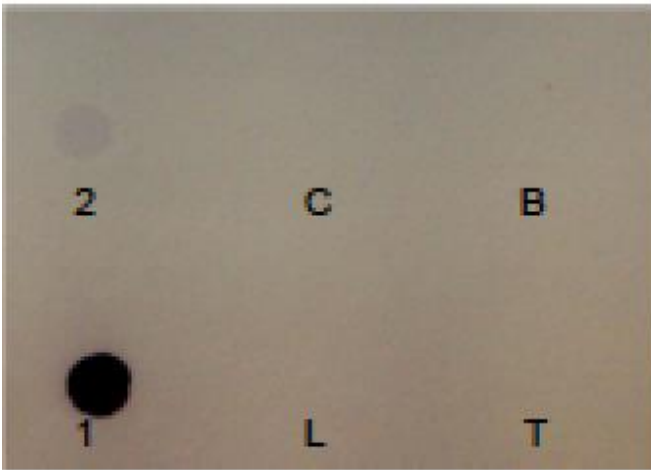


Figure 3.

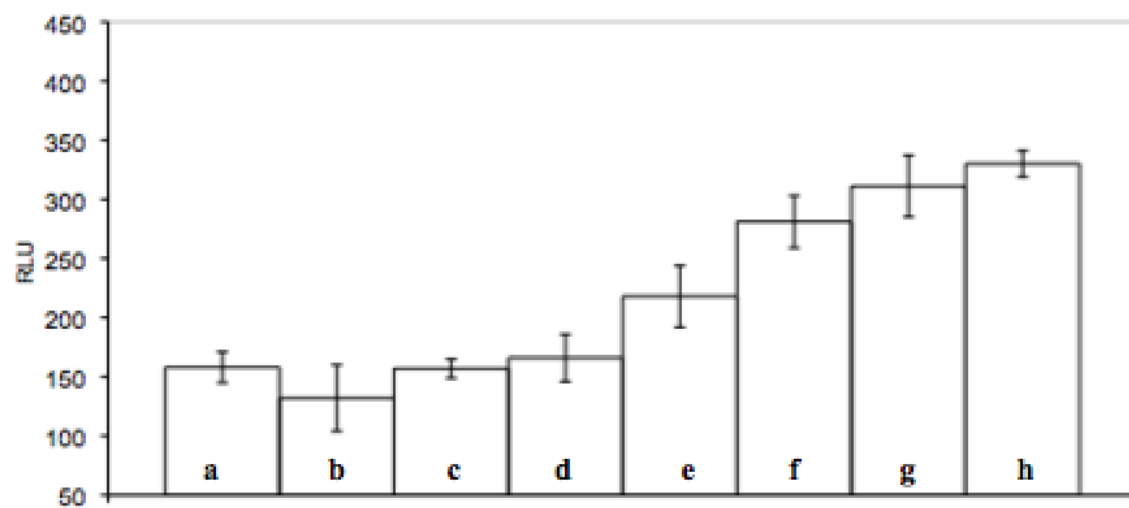


Figure 4.

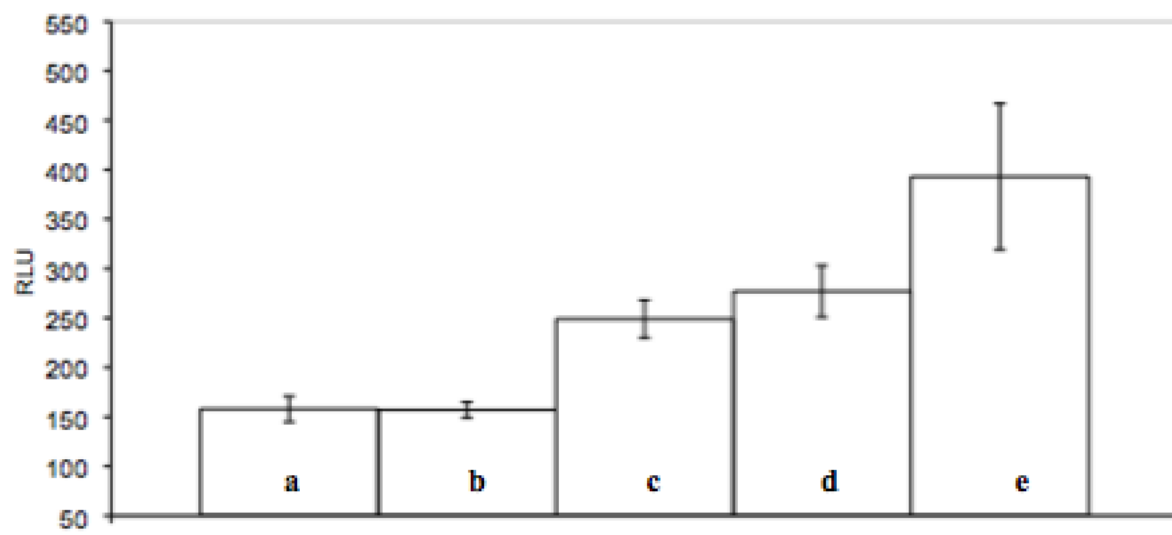


Figure 5.

Table 1. Correspondence between the percentages of the pork meat and the real amount of pork meat used for the DNA extractions using the protocol with lysing buffer.

Sample	Content of pork meat in 1 mL Stomacher bag homogenate	Amount of meat sample used in the extraction using lysing buffer
100% pork	200 mg	200 ng/μL
10% pork	20 mg	20 ng/μL
5% pork	10 mg	10 ng/μL
1% pork	2 mg	2 ng/μL

Table 2. Correspondence between RLU obtained by fibre optic analysis and the amount of pork meat present in the 20 µL samples used in the tests, P (pork), B (Beef).

Sample	pork (pg)	RLU	SD*
no DNA no probe	0	150	13
no DNA plus probe	0	150	8
1%P-99%B	8.6	250	19
5%P-95%B	43	275	26
10%P-90%B	86	400	74

*SD: standard deviation