Elsevier Editorial System(tm) for Biosensors and Bioelectronics
Manuscript Draft

Manuscript Number:

Title: OLED-based DNA biochip for Campylobacter spp. detection in poultry meat samples

Article Type: Full Length Article

Section/Category: The others

Keywords: organic light emitting diode (OLED), Campylobacter spp., poultry meat, DNA biochip

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Dear Editor,

I enclose the manuscript “OLED-based DNA biochip for *Campylobacter* spp. detection in poultry meat samples" by Marisa Manzano, Francesca Cecchini, Marco Fontanot, Lucilla Iacumin, Giuseppe Comi and Patrizia Melpignano, which I would like to submit to your attention for publication in Biosensors and Bioelectronics.

The present paper describes the creation of a genosensor built using an organic light emitting device (OLED) and a new designed specific DNA probe labelled with a fluorophore, for possible application in point of care diagnostic systems. This is the first time the OLED technology is coupled to fluorophore labelled DNA sequences to detect a bacteria. This application describes the utilisation of the genosensor for the detection of *Campylobacter spp.*, responsible for zoonosis with fatal episodes due to its presence mainly in poultry meat samples. The official plate count method (ISO 10272-1B: 2006), the Polymerase Chain Reaction, the dot blot and the proposed OLED system, were also used to evaluate the presence of *Campylobacter spp.* in poultry meat samples. Classical and molecular methods were used for validation of the results obtained by the OLED. The genosensor got great results being able to reduce time requested for meat analysis and having very high sensitvity as well. The results can be achieved within 24 h and the sensitivity was 20 fold higher the sensitivity of the blotting method or PCR. The optimized genosensor could be useful for application on poultry meat sample analyses in food industries and slaughterhouses to reduce the risk for commercialization of contaminated meat, but it can also be used for the detection of various pathogens in food samples using different DNA probes.

In our opinion this manuscript is coherent with the aims of Biosensors and Bioelectronics.

The results of this research have never been submitted for publication anywhere before and we hope that you find our work of interest for your readers and expect to hear from you soon.

Best regards

Marisa Manzano

OLED-based DNA biochip for *Campylobacter* spp. detection in poultry meat samples

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Abstract

Integrated biochips are the ideal solution for producing portable diagnostic systems that uncouple diagnosis from centralised laboratories. These portable devices exploit a multi-disciplinary approach, are cost effective and have several advantages including broader accessibility, high sensitivity, quick test results and ease of use. The application of such a device in food safety is considered in this paper. Fluorescence detection of a specific biological probe excited by an optical source is one of the most commonly used methods for quantitative analysis on biochips. In this study, we designed and characterised a miniaturised, highly-sensitive DNA biochip based on a deep-blue organic light-emitting diode. The molecular design of the diode was optimised to excite a fluorophore-conjugated DNA probe and tested using real meat samples to obtain a high sensitivity and specificity against one of the most common poultry meat contaminants: *Campylobacter* spp.. Real samples were analysed also by classical plate methods and molecular methods to validate the results obtained by the new DNA-biochip. The high sensitivity obtained by the OLED based biochip (0.37 ng/ml) and the short time required for the results (about 24 h) indicate the usefulness of the system.

Keywords: organic light emitting diode (OLED), *Campylobacter* spp., poultry meat, DNA biochip

1. Introduction

Early and accurate diagnosis is very important for food security, especially before the food is distributed for human consumption. Currently one of the most commonly used diagnostic methods, the plate count method of grown microorganisms, requires a long time during which some food that contains pathogens may be distributed and consumed. Moreover, some bacteria can be stressed by food industry heat treatments and are not able to grow on selective agar plates, (viable but-not culturable, VBNC), thus making plate count methods unsuitable for testing. Molecular biology has greatly improved the techniques by reducing the time required to obtain results. Although real-time-PCR (Levi et al., 2003) allows results to be obtained in a few hours, the inhibition caused by contaminants in the DNA polymerase used in PCR, can produce false-negative results. Moreover, PCR tests are normally run in a laboratory context, while the opportunity to perform point-of-care food controls can improve the safety of food distribution. Recent advances in biosensor technology promise sensitive and specific point-of-care tests with rapid results. Optical sensors (Passaro et al., 2012), acoustic sensors (Jia et al., 2012), microwire sensors (Lu and Jun, 2012), microfluidic based sensors (Yager et al., 2006) and electrochemical biosensors (Marks et al., 2007) offer highly sensitive and fast devices that can be used for the rapid screening of foods to detect foodborne pathogens prior to distribution. Antibodies, cells and DNA have been used as probes in the fabrication of biosensors (Lei et al., 2006). In particular, DNA is a biological element that is useful for the creation of genosensors (DNA-biosensors) (Cecchini et al., 2012), which allow the rapid monitoring of hybridisation with the target DNAs. These biosensors, which are based on the oligonucleotide sequences chosen as probes, are specific and sensitive. To reveal the presence of a hybrid generated by the annealing of the DNA probe to the DNA target in various samples, it is possible to use a detected a fluorescent signal. In particular, DNA probes can be labelled with fluorophores, and their weak optical

signal can be detected using a CCD (charge-coupled device) camera after a suitable optical excitation. The utilisation of an OLED (organic light emitting device) source as a fluorescence excitation source, to produce a sensitive biochip has been demonstrated by different authors, (Yao, et al., 2005; Hofmann et al., 2005; Pais et al. 2008; Ramuz et al., 2009; Lamprecht, 2010). In particular, the use of an optimised OLED source for the detection of protein arrays has been demonstrated by Marcello et al., (2013). In this work we apply, for the first time, this OLED light source to a DNA-biochip for the detection of *Campylobacter,* one of the most important pathogens that is responsible for human gastroenteritis, which still causes large economic losses worldwide. The European Food Safety Authority (EFSA) estimated that approximately nine million cases of campylobacteriosis occur each year in Europe, with a cost to public health systems of approximately EUR 2.4 billions. Campylobacteriosis is considered the most frequent foodborne illness in the European Union (EU), and the most common food contaminated by *Campylobacter* is chicken meat. Classical methods for *Campylobacter* identification in food samples rely on broth enrichment and colony growth on selective agar plate which takes, at least five days (ISO 10272-1B: 2006)(Revision ISO 10272, 2010). In this work, we tested the sensitivity of a new bio-sensor using both pure culture reference strains and real poultry meat samples to determine the sensitivity of the bio-chip. The tests results are compared with the standardised laboratory methods including PCR, broth enrichment and colony growth, to demonstrate the relevance of this system for a rapid, simple and reliable point-of-care test for poultry meat.

2. Materials and methods

2.1 OLED Fabrication

For this experiment high quality polished borosilicate glass substrates of 1 mm thick coated with 150 nm of indium tin oxide (ITO) of about 20 Ohm/square surface resistance have been

used. The ITO has been partially removed by a lithographic process using UV curable resins and a mask aligner in a class-10 clean room. Before coating the samples with poly(3,4-ethylenedioxythiophene) - poly(styrenesulfonate) (PEDOT-PSS), an ultrasonic cleaning with organic solvents (Acetone, Iso-Propanol and Ethyl Alcohol) and de-mineralized water has been performed on each substrate. All samples have then been dried with nitrogen. After cleaning, the PEDOT-PSS was deposited on the ITO at a speed of 2000 RPM for 20 sec in the clean room. After the PEDOT-PSS coating, the samples were annealed at 100°C in air atmosphere for 5 min. After this treatment the samples were transferred in a BOC EDWARDS 500 evaporator, integrated in a pure nitrogen filled JACOMEX glove box, for both the organic layers and the metal cathode deposition. For the organic layers deposition Knudsen cells, each integrated with a thermocouple, for a PID (proportional integral derivative control) setting and a constant monitoring of the cell temperature, have been used. The deposition rate of the organic layers was set at 0.1 nm/sec and the film thickness was monitored, during the evaporation, by a calibrated quartz microbalance. A thin LiF layer (1 nm) and a pure aluminum (99.99%) layer was then deposited by electron beam technique in the same BOC EDWARDS 500 evaporator. Two different evaporation rates of 0.01 nm/sec and 0.2 nm/sec were respectively used for the thin films evaporation and, also in this case, the film thickness has been monitored by a calibrated quartz microbalance. During both the organic and metal evaporations the pressure in the vacuum chamber was maintained at 1×10^{-6} mbar. After the evaporation the organic light emitting diode (OLED) samples have been encapsulated with a glass lid and a UV curable resin in the glove box. The optical and electrical characterization of the OLED samples was performed in air. The spectral emission and the radiance of the OLED device, measured at normal incidence, was recorded with a GL Spectis 5.0 spectroradiometer (GL Optics GmbH), while the J-V curves of the OLED device were recorded with a source meter specifically developed at LAPLACE laboratory

(Toulouse, France).

2.2 Strains selection and DNA preparation

As a first step for the bio-chip construction, 32 microorganisms (31 bacteria and 1 yeast from international collections) listed in Table 1 were used for testing the specificity and sensitivity. The DNA of the reference strains was extracted and purified from one millilitre of overnight broth culture using the Wizard® Genomic DNA Purification Kit (Promega, Milan, Italy) (Cecchini et al., 2012). The nanodrop 2000c (Thermo Fisher Scientific, Wilmington, DE, U.S.A.) was used to evaluate the purity and concentration of the DNA samples.

2.3 DNA probe construction and test

A new 55-base DNA detection probe (CampyDet) (5'CACTTTTCGGAGCGTAAACTCCTTTTCTTAGGGAAGAATTCTGACGGTACCTA AG - 3') specific for the 16S rRNA gene of *Campylobacter* spp. was designed, to be used with the 45-base capture probe (5'-GGGAGAGGCAGATGGAATTGGTGGTGTAGGGGTAAAATCCGTAGA-3') (Fontanot et al., 2014) in the biochip system.

The DNA sequences retrieved from GenBank using the following accession numbers: HM007568.1, DQ174142.1, DQ174141.1 for *Campylobacter jejuni*, HM007569.1, AB542728.1, JX912505.1 for *C. coli*, GQ167657.1, AF550634.1 for *C. lari*, and DQ174157.1, AF497805.1, GQ167658.1 for *C. upsaliensis*, AY277975.1 for *Helicobacter ganmani* CCUG 43527, and AY277974.1 for *Helicobacter ganmani* CCUG 43526 were analysed.

The probes were tested in silico using Blast (http://blast.ncbi.nlm.gov/Blast.cgi), before being used in bio-chip construction. The CampyDet probe, after the labelling of the 5' end with

digoxigenin (Dig-CampyDet probe), was tested using the dot blot technique (Fontanot et al., 2014) on the synthetic ssDNA sequence, complementary to the CampyDet probe, to evaluate the DNA probe sensitivity and to establish the optimal concentration of the labelled probe for use in the experiments. The DNA extracted from the reference strains listed in Table 1 according to Manzano et al. (2003) was also used in the dot blot procedure, to test the specificity of the probes. Moreover, DNA from *Helicobacter suis* 19735 (DSM), *Arcobacter cryaerophilus* 7289 (DSM).

2.4 Silanization of the glass slides and capture-probe binding

As support of the biochip, microscope cover glasses (28 x 19 mm) were used. For silanization the protocol described by Marcello et al. (2013) was followed with some modifications. The cover glasses were treated with 10% NaOH (2.5 mM, Sigma, Italy) at room temperature for 1 h, rinsed with deionized water and treated with 0.1 N HCl for 15 min. After a washing step with deionized water, the glass slides were rinsed in acetone and dried at 50°C for few min, and immersed in a 0.5 % APTES (3 aminopropyltriethoxysilane) (Fluka, Milan, Italy) solution in deionized water for 30 min at room temperature. Slides were then rinsed three times in deionized water followed by 10 min washing under shaking, dried at 160°C for 1 h and cooled at room temperature for 30 min. After modification at 5' end with an amino group instead of the digoxigenin (amino-capture-probe), 1 µl of the amino-probe at 100 ng/µl, in triplicate, were drop off on each glass slide and incubated at 4°C overnight to bound to microscope cover glass surface.

2.5 Labelling of the detection probe by Alexa Fluor® 430

For the first time the fluorophore Alexa Fluor® 430 (Invitrogen, Monza, Italy) that exhibits the absorption between 400 - 450 nm and fluorescence emission beyond 500 nm, was bound

to a DNA molecule and subsequently used in a biochip detection system.

250 µg of Alexa Fluor® 430 was mixed with 14 µl of DMSO (Dimethyl sulfoxide)(Sigma, Milan, Italy), then 7 µl of nuclease free water, 75 µl of sodium tetraboroidrate 0.1M (pH 8.5) and 4 µl of the CampyDet probe at 25 µg/µl, with an amino group at 5' end, were added. The tube was incubated overnight at room temperature under agitation for 2 h. After incubation NaCl 3 M in order to 1:10, and 2.5 cold absolute ethanol, were mixed in the tube and incubated at -20°C for 30 min. The tube was then centrifuged at 12,000 xg for 30 min. The supernatant was discarded and the pellet washed two times with cold ethanol at 70%, dried for few min, resuspended in 200 µl of 50% formamide and kept at 55°C for 5 min. 20 µl of the probe with 5 µl of GLB (Gel Loading Buffer) were loaded into an agarose gel at 2% for purification. The band containing the labelled probe (Alexa-CampyDet) was cut out from the gel, soaked in sterile deionized water overnight at 4°C. The eluted Alexa-CampyDet probe was collected in a new tube and maintained at -20°C till utilization.

2.6 Measurements and image processing

For all the tests the fluorescence signal was acquired using a high sensitive camera (Hamamatsu Orca C8484-03G02) integrated with a microscope objective. The images were acquired with the gain set to 1 and with an integration time of 30 sec. The image digitalization was set at 12 bit with a gray scale ranging from 0 to 4095. The image processing was performed with the free software ImageJ (http://rsb.info.nih.gov/ij/, 1997-2009). A process of de-speckle to eliminate scattering luminous spots have been applied to all the images as well as a threshold adjustment to identify the emitting areas. Mean intensity of the identified area was measured and background subtracted, analysis and plotting was performed in R (R Development Core Team, Computing R. F. f. S. , Ed., Vienna, Austria, 2010).

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186 *2.7 Construction of the bio-sensor sensitivity curve*

187 The characterised DNA probes were then used for the bio-sensor construction.

188 A bottom-emission small molecule-based OLED, which was optimised to obtain a deep-blue
189 (DB) colour emission with a peak wavelength of 434 nm (DB-OLED- Marcello), was used to
190 excite the fluorescence of the commercial dye Alexa Fluor® 430 (Invitrogen, Monza, Italy),
191 with the absorption peak located at 434 nm and the emission peak located at 541 nm
192 (Panchuk-Voloshina et al., 1999). An in-depth physical description and characterisation of
193 the patented OLED, adopted in this bio-sensor, using the fluorescent molecule, a-NPD [N,
194 N'-diphenyl-N, N'-bis (1-naphthylphenyl)-1, 1'-biphenyl-4, 4'-diamine] as an emitter, has
195 been reported by Marcello et al. (2013).

196 One hundred-micrometre-thick silanised microscope cover glasses (28 x 19 mm) were used
197 as support of the bio-probes employed in the biochip. The DNA capture probes after a
198 modification at the 5' end by adding an amino group instead of the digoxigenin (amino-
199 capture-probe), were deposited on the silanised glasses. A 0.5 µl drop of the amino-capture-
200 probe at 100 ng/µl was deposited on each glass slide and incubated at 4°C overnight to bind
201 to the glass surface.

202 The glass slides with the bound amino-capture probe were washed twice in deionised water
203 prior to utilisation. Then, 0.5 µl of the DNA samples and 0.5 µl of the Alexa-CampyDet
204 probe (CampyDet probe labelled at the 5' end with the Alexa Fluor® 430 dye) were spotted
205 on each slide in a ratio 1:4 after 5 min of denaturation at 95°C. A sensitivity curve was
206 determined using different concentrations of DNA (25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.12
207 ng/ml, 1.50 ng/ml, and 0.75 ng/ml) from *C. jejuni* subsp. *jejuni* ATCC 49943, and various
208 concentrations (100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.12 ng/ml, and 1.5
209 ng/ml) of the Alexa-CampyDet probe. The microscope cover glasses were incubated at 63°C

for 1.5 h in a sterile petri dish to prevent evaporation, and they were washed twices in sterile deionised water to eliminate the unbound DNA and the unbound Alexa-CampyDet probe. Finally, the glass slides were assembled on a rectangular DB-OLED together with a high-pass optical excitation filter with a high extinction at the wavelength corresponding to the fluorophore emission (transmission (T) < 10^{-5}) and a high transmission in the excitation spectral region. A second bandpass filter centred on the fluorophore wavelength emission was used before the signal capture camera. The DB-OLED was used at 7.0 Volts with a total optical energy density of 85 mW/cm^2 (Banerjee et al., 2010). The fluorescence signal was acquired with a high sensitivity CCD camera, acquiring an image with a 12-bit digitalisation. The CCD gain was set at the maximum value and an integration time of 30 sec was used for image acquisition. For the analysis, the background of each image was subtracted: a de-speckle algorithm was used, and the images so obtained were thresholded to automatically identify and measure the circular spots. To calculate the sensitivity curve, the mean value of the spot was considered and the results are reported in Fig.1.

2.8 Real samples analyses by classical plate method, molecular methods and the OLED biochip

After the determination of the sensitivity curve, an experiment using real poultry meat samples was performed. Seven poultry samples were collected from local markets and analysed for the presence of *Campylobacter* spp. according to the official method ISO 10272-1B: 2006 (Revision ISO 10272, 2010) and by direct plating on the selective modified Charcoal-Cefoperazone-Deoxycholate Agar (mCCDA) (Oxoid, Milan, Italy). Samples were also evaluated for the enumeration of mesophilic aerobic microorganisms and *Enterobacteriaceae.*

Twenty-five grams of skin from each poultry sample was transferred to a filter sterile

Stomacher bag (PBI, Milan, Italy); 100 ml of saline-peptone water was added (8 g/l NaCl, 1 g /l bacteriological peptone, Oxoid, Milan, Italy), and the contents were mixed for 1.5 min. Aliquots of 0.1 ml were used to obtain the mesophilic aerobic count on Plate Count Agar (PCA, Oxoid) and to count *Campylobacter* spp. on mCCDA, whereas aliquots of 1 ml were used for the enumeration of *Enterobacteriaceae* on Violet Red Bile Glucose (VRBG) Agar (37°C for 24 h). DNA was extracted from 10 ml saline-peptone water and used for the homogenisation of the samples, purified as described by Manzano et al. (2003); these samples were used in the molecular methods reported and in the OLED biochip system proposed in this work. Then, 0.5 µl of the DNA extracted from the physiological solutions used to homogenise each chicken sample, and standardised at 25 ng/µl, was mixed with 0.5 µl of the Alexa-CampyDet probe (Alexa Fluor® 430 labelled) at 100 ng/µl and spotted (in triplicate) onto the glass slides holding the amino-capture probe. The glass slides were placed on the OLED, and the fluorescence was measured by the CCD camera using the acquisition parameters described above. The fluorescence value reported for each sample was obtained as an average value of three measurements. DNA was also extracted from the Preston enrichment broths after 48 h, purified and used in dot blot and PCR assays.

The colonies grown on mCCDA and suspected to be *Campylobacter* based on morphology were isolated and tested for motility, oxidase and catalase activity and growth at 25°C, Gram stained and assayed by PCR using the protocol proposed by Fontanot et al. (2014). Amplicons obtained by PCR were sent to Eurofins MWG Operon (Eurofins MWG Operon, Ebersberg, Germany) for sequencing.

3. Results

3.1 specificity and sensitivity of the DNA probes

The sensitivity of 20 pmol/ml digoxigenin-labelled capture probe was determined by dot blot

to be 1 ng/ml using the synthetic ssDNA oligonucleotide sequence complementary to the probe as the target, and 25 ng/ml using the genomic DNAs extracted from the *Campylobacter* reference strains reported in Table 1 as the target. The probe showed high specificity under the conditions proposed in the described protocol, annealing only to the *Campylobacter* species used as reference strains. The sensitivity of the 55-base Dig-CampyDet probe (20 pmol/ml) was determined by dot blot to be 1 ng/ml using the ssDNA complementary sequence and 25 ng/ml using pure culture reference strains as the target DNA.

The sensitivity curve obtained using the OLED biochip is reported in Fig. 1. The serial dilutions of the tested DNA is plotted against the number of counts measured in the luminous spots (count range between 0 and 4095). The results obtained by the biochip system using various concentrations of DNA from *Campylobacter* pure culture show an excellent linearity ($R^2 = 0.99$). The linear regression interpolation parameters are also reported in Fig. 1.

As observed from Fig. 1, the OLED biochip, which included both the amino-capture-probe and the Alexa-CampyDet probe, showed a sensitivity of at least 0.37 ng/ml, as obtained by extrapolating the sensitivity curve, which is almost two orders of magnitude higher than the sensitivity obtained with the dot blot method (25 ng/ml). Moreover, the efficacy of the method in preventing false negative results was evaluated using various negative samples: a) no capture probe (omission of the amino-capture probe); b) no DNA (omission of the DNA sample); and c) no Alexa (omission of the Alexa-CampyDet probe). These control experiments confirmed the specificity of the test (images not shown). The images obtained (in triplicate) using the DNA of *Campylobacter* (a positive sample) at a concentration of 6.25 ng/ml show a high level of fluorescence, whereas the images obtained using DNA of *E. cloacae* (a negative sample previously tested by dot blot as well) at the same concentration do not show any fluorescence signal, as reported in Fig. 2.

285 *3.2 real poultry meat samples analyses results*

286 The results of the classical plate count, PCR, dot blot and OLED biochip tests are reported in
287 Table 2. *Campylobacter* spp. were detected in four of the seven chicken samples analysed by
288 direct plating onto mCCDA and varied from 15 CFU/g (colony forming units per gram) to
289 3.6 x 10^3 CFU/g, whereas three samples were below the detection limit of the method (< 5
290 CFU/g).

291 Samples 1, 2, 4 and 7, which exibited growth of *Campylobacter* on the selective medium
292 mCCDA both, after direct plating from the homogenisation solution, and after the enrichment
293 step in Preston broth (isolates), were also positive for *Campylobacter* according to PCR, dot
294 blot and the OLED biochip analysis.

295 In fact, isolates from mCCDA were confirmed to be *Campylobacter* spp. by motility test,
296 Gram staining, oxidase activity, catalase activity and growth at 25°C. The DNA sequences
297 corresponding to the amplicons produced by PCR, obtained from Eurofins MWG Operon
298 centre, matched 100% the *Campylobacter* sequences retrieved from GenBank using Blast
299 (http://blast.ncbi.nlm.nih.gov/Blast.cgi), thus confirming the identification of *Campylobacter*
300 obtained by PCR (Table 2). The blue spots obtained for the samples 1, 2, 4 and 7 through the
301 hybridisation of the specific digoxigenin-labelled probe used in the dot blot with the DNAs
302 extracted from the Preston broths confirmed the presence of *Campylobacter*.

303 In addition, the OLED biochip analysis confirmed the positivity of the samples 1, 2, 4 and 7.
304 In fact, the luminosity values obtained in the positive samples are consistent with the results
305 obtained for the sensitivity curve (Fig. 1). The images of two samples, i.e., the positive
306 chicken meat sample number 2, and the negative chicken meat sample number 3, which were
307 analysed using the DNA extracted from the physiological solutions, thus without any
308 enrichment step in broths, are reported in Fig. 3.

309 A cut-off value of luminescence count was set at approximately 500 AU, which corresponds

310 to a negative meat sample in all of the other tests (the ISO 10272-1B: 2006 (no colony
311 growth onto selective agar plates), PCR (no amplicons obtained) and dot blot (no blue
312 spots)), to allow the discrimination between positive and negative samples. For this reason,
313 samples below this value (3, 5 and 6) were considered negative. The values between 0 and
314 500 AU, which were obtained for some negative real meat samples, could be due to the
315 presence of dead *Campylobacter* cells, which are able to anneal the specific Alexa-CampyDet
316 probe and produce a weak luminescence that is detectable due to the high sensitivity of the
317 system. The mesophilic aerobes varied from 3.0 $x10^4$ to 5.9 x 10^8 CFU (colony forming
318 units)/g, and those of *Enterobacteriaceae* varied from 1.8 x 10^3 to 3.3 x 10^7 CFU/g. The
319 microbial contamination evaluated on PCA and VRBG indicates that the presence of
320 *Campylobacter* is not affected by the hygienic level of the samples; in fact, it can be present
321 both in low and high levels of sample contamination. A systematic control for the presence of
322 *Campylobacter* spp. should also be performed in meat companies that have a satisfactory
323 level of hygiene during meat samples processing.
324
325 **4. Discussion**
326 The detection and identification of *Campylobacter* spp.-contaminating poultry meat samples
327 is usually carried out by culturing techniques that are laborious and time consuming. The
328 utilisation of molecular methods such as PCR and dot blots allow for the faster detection of
329 *Campylobacter* spp. in food samples, because these methods can be applied to DNA
330 extracted from the Preston enrichment broth without requiring cell growth (Silva et al., 2011).
331 Moreover, molecular techniques are able to detect the VBNC (Oliver, 2005) forms of
332 *Campylobacter* spp. that often do not grow on selective media due to the stressing conditions
333 of food, although they are present in food. Comparing the time required to obtain results, we
334 can assert that the molecular methods used are convenient in comparison with plate count

335 methods because they are able to give results within 48- 72 h, whereas classical
336 microbiological methods require approximately one week. Due to its sensitivity, the OLED
337 biochip proposed is even more rapid, and takes only 24 h.
338 Moreover, the OLED biochip was able to detect *Campylobacter* using the DNA that was
339 extracted directly from the physiological solutions used for the homogenisation of the
340 chicken meat samples. Thus, it allows for the first time, for *Campylobacter* spp. to be
341 detected without any prior enrichment step in Preston broth, a step that is necessary for
342 obtaining sensitive results in the current commercial ELISA tests and qPCR assays. The
343 OLED biochip reached a sensitivity of 0.37 ng/ml DNA, which was approximately 20-fold
344 higher than the sensitivity obtained with dot blot assay (25 ng/ml), when performed under the
345 same conditions using DNA extracted from the reference strains listed in Table 1. The
346 detection of *Campylobacter* in a chicken sample by dot blot requires a 48-h enrichment step
347 in Preston broth to allow *Campylobacter* cells to multiply and to reach a number detectable
348 by this method, whose sensitivity is 20-fold lower than the OLED biochip sensitivity. The
349 OLED biochip system is also highly specific as demonstrated by the analysis made using the
350 DNA probes on the various microorganisms reported in Table 1, considering that only
351 *Campylobacter* DNAs gave positive results using the two DNA probes designed.
352 The ability of the biochip to measure real meat samples has also been demonstrated by the
353 absolute correlation obtained with the standard methods used to analyse the poultry meat
354 samples to detect *Campylobacter* spp., as shown in Table 2, when applying a minimum AU
355 value (cut-off value) of approximately 500 counts. Based on this assumption, the same
356 samples positive by the OLED biochip were alo positive by the other methods used,
357 including the direct plate count on mCCDA, which shows a minimum level of detection of 5
358 CFU/g. This correlation among the different methods adopted for the analysis validated the
359 obtained results. Another very important consideration is that this method of analysis

preserves the fluorophore functionality after the first analysis. The low optical energy density used to excite the fluorescence (approximately 85 mW/cm^2) does not produce a photobleaching effect on the dyes, as is common in other optical tests that uses high optical energy density from unoptimised sources (laser or high power LED). Due to the long stability time of the used and undamaged fluorophore, the proposed OLED biochip is a non-destructive assay. If stored at 4°C in a dark environment, the biochip can be reused after months, giving nearly the same fluorescence values when measured immediately after its preparation, and excited with the same optical energy density. Two fluorescence images of the same sample (*Campylobacter* DNA at 6.25 ng/ml), measured at the first sample analyses and one month later, after storage at 4°C in a dark place, are shown in Fig. 4. The measured fluorescence taken after one month, with a different OLED source but at the same optical energy density, shows a difference of less than 5% compared with the value acquired immediately after the preparation. The stable biochip allows the storage of the samples analysed for possible successive checks thereby confirming the robustness of the proposed system.

5. Conclusions

The OLED-based DNA biochip proposed in this paper is: i) higly sensitive, at least 20-fold more than the classical and molecular methods previously developped; ii) easy to use; iii) stable, as samples can be analysed after one month without variations in the measures.

Acknowledgements

The work at OR-EL.doo was partially supported by Slovenski Podjetniski Sklad under the contract SIA10/00020. PM acknowledge support from the FP7 MNT ERA OLED-CHIP Consortium.

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Figure 1: Sensitivity curve obtained using different DNA concentrations. The mean number of counts (ranging from 0 to 4095) of the fluorescent signals, recorded with a 12 bit digitalization, is reported as a function of DNA concentration. Different images of the obtained fluorescence signals are shown: a) DNA concentration of 12.5 ng/ml; b) DNA concentration of 6.25 ng/ml; c) DNA concentration of 3.12 ng/ml; d) DNA concentration of 1.5 ng/ml; and e) DNA concentration of 0.75 ng/ml. For clarity at the last image is associated its thresholded image. The DNA concentration of 25 ng/ml produced a signal in saturation not reported in the sensitivity curve. The measured fluorescence signals present a linear behaviour as a fuction of DNA concentration (R^2 = 0.99).

Figure 2: Fluorescence images obtained hybridizing at 63°C 6.25 ng/ml DNA (in triplicate) extracted from pure cultures of two different bacteria. The fluorescent signal present for *Campylobacter jejuni* (a) indicates a positive result (around 1500 AU), whereas the absence of fluorescence showed for *Enterobacter cloacae* (b) indicates a negative result (< 10 AU). In both the measures of the two images the background was subtracted.

Figure 3: Fluorescence images (in triplicate) obtained with DNA extracted from physiological solutions used for homogenising two chicken meat samples (without enrichment process), after the background subtraction. Sample 2 (a) showed an intense fluorescence signal indicating the presence of *Campylobacter* (> 3400 AU), and thus considered positive. Sample 3 (b) showed a low fluorescent signal, lower than the cut-off limit (< 500 AU), indicating the absence of *Campylobacter* and thus considered negative. These results were confirmed by PCR, dot blot and plate count method.

Figure 4: Comparison of the fluorescent signal obtained by the same sample of DNA of *Campylobacter jejuni* at a concentration of 6.25 ng/ml measured just after the sample preparation (a) and one month later (b), after background subtraction. The two samples have been measured with two different OLEDs at the same optical power density (85 mW/cm^2) giving the following results: a) 1450 AU and b) 1380 AU.

Table 1: Reference microorganisms used to test sensitivity and specificity of the DNA probes.

N°	Microorganism	Source
1	*Weissella cibaria*	*DSM 14295
2	*Vibrio spp.*	*DSM 14379
3	*Escherichia coli*	°DISTAM
4	*Pseudomonas aeruginosa*	°DISTAM
5	*P. migulae*	°DISTAM
6	*P. fluorescens*	°DISTAM
7	*P. brennerii*	°DISTAM
8	*Bacillus coagulans*	*DSM 2308
9	*B. subtilis*	*DSM 1029
10	*B. cereus*	*DSM 2301
11	*Proteus vulgaris*	°DISTAM
12	*Yersinia enterocolitica*	°DISTAM
13	*Morganella morganii*	°DISTAM
14	*Salmonella enteritidis*	*DSM 4883
15	*Listeria monocytogenes*	§ATCC 7644
16	*Citrobacter freundii*	*DSM 15979
17	*Enterobacter cloacae*	*DSM 30054
18	*Aeromonas sobria*	*DSM 19176
19	*Lactobacillus plantarum*	*DSM 20174
20	*Pediococcus pentosaceus*	*DSM 20336
21	*Leuconostoc lactis*	#CECT 4173
22	*Saccharomyces cerevisiae*	§ATCC 36024

23	*Campylobacter jejuni subsp. jejuni*	*DSM 4688
24	*C. jejuni*	§ATCC BAA-1153
25	*C. jejuni*	§ATCC 49943
26	*C. coli*	*DSM 24155
27	*C. coli*	*DSM 24128
28	*C. coli*	§ATCC 43478
29	*C. lari subsp. lari*	*DSM 11375
30	*C. upsaliensis*	*DSM 5365
31	*Helicobacter pylorii* p1	^Hospital of Udine
32	*Helicobacter pylorii* p2	^Hospital of Udine

479 §ATCC: American Type Culture Collection (Manassas, VA, USA)

480 °DISTAM: Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (Milan,
481 Italy)

482 *DSM: Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH (Braunschweigh,
483 Germany)

484 #CECT: Colección Española de Cultivos Tipo (University of Valencia, Spain)

485 ^: isolated from hospitalized patient (Hospital of Udine, Italy)

Table 2: Data of the microbial evaluation of 7 chicken samples using: plate count method (PCA, VRBG and mCCDA) (values expressed in colony forming units (CFU) /g); PCR, dot blot, and the OLED bio-chip (results expressed as presence (+) or absence (-)). OLED was used on samples from physiological solution immediatly after homogenization, whereas PCR and dot blot on DNA extracted from Preston broth after 48 h enrichment.

Sample	mCCDA[§§]	PCR*	PCA[§]	VRBG°	Preston broth °°	OLED**
				isolates	dot blot	
1	1.5×10^1	+	4.0×10^4	5.3×10^3	+	+	+
2	1.5×10^1	+	6.5×10^7	6.0×10^5	+	+	+
3	< 5	-	3.6×10^6	1.8×10^3	-	-	-
4	3.6×10^3	+	3.1×10^8	7.0×10^5	+	+	+
5	< 5	-	3.7×10^7	3.9×10^5	-	-	-
6	< 5	-	5.9×10^8	3.3×10^7	-	-	-
7	1.6×10^3	+	3.0×10^4	2.7×10^3	+	+	+

[§§] (modified Charcoal-Cefoperazone-Deoxycholate Agar); [§] (Plate Count Agar); ° Violet Red

Bile Glucose Agar;

* PCR was performed according to the protocol described by Fontanot et al. (16);

°° positivity was assigned when *Campylobacter* colonies were present onto the selective media (isolates), and when the blue spot due to the annealing of the specific probe was obtained;

** positivity was assigned when AU (Arbitrary Units) values were above 500 AU

Figure-1 Manzano

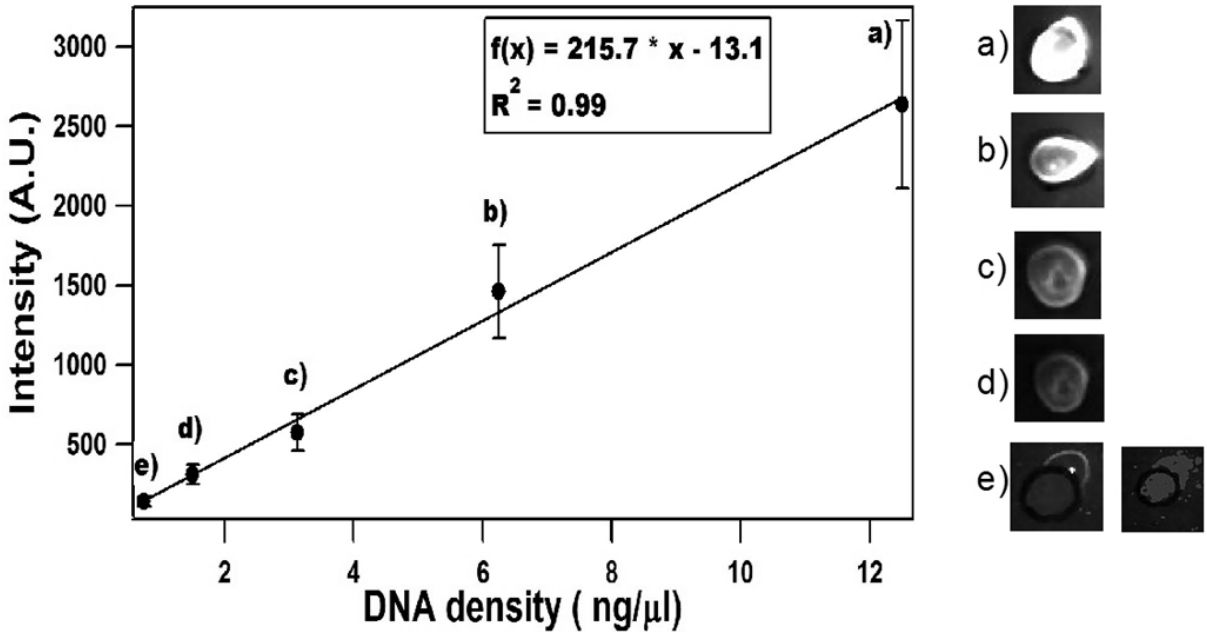


Figure-2 Manzano

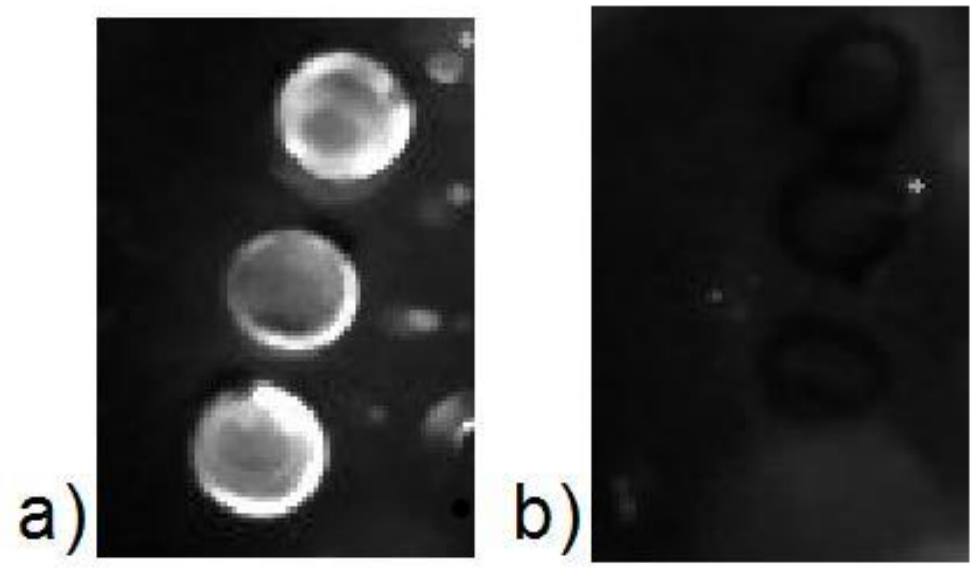


Figure-3 Manzano

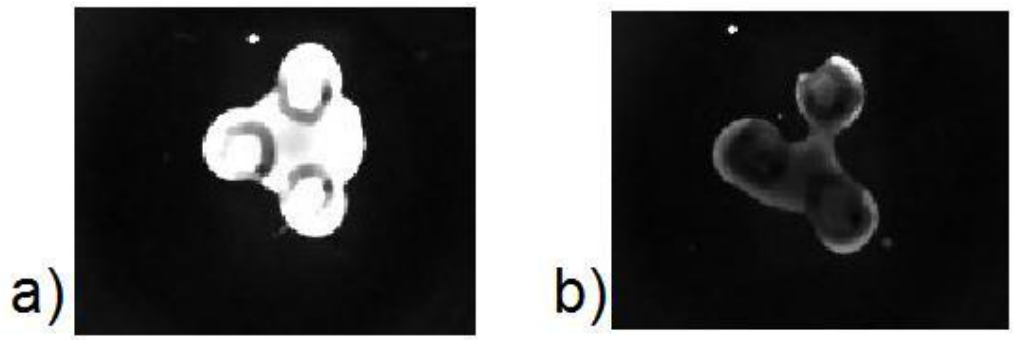
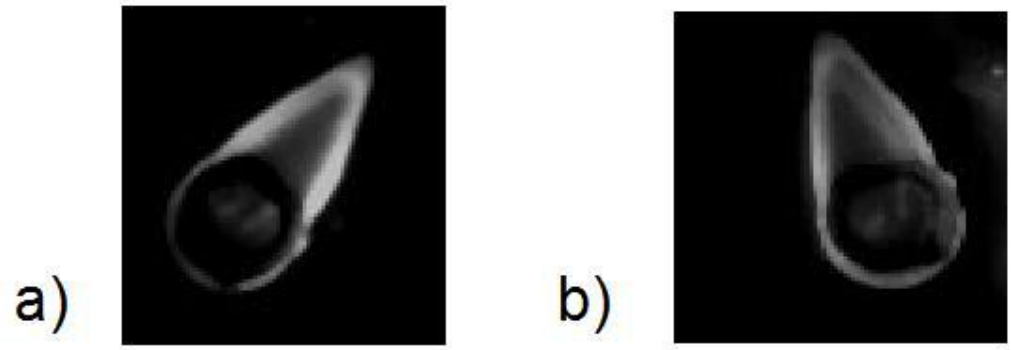


Figure-4 Manzano



***Highlights (for review)**

- A DNA OLED based biochip was developped for pathogen detection
- The biochip was able to measure real meat samples showing high sensitivity
- The proposed OLED biochip is a non-destructive assay