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On-line detection system of Escherichia coli O157:H7: A new support for Public Health

Original Article

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SUMMARY.

Introduction. Motivated by countless cases of diarrheic infections occurred in developing countries, an intensity-modulated fiberoptic biosensor was calibrated using *Escherichia coli* O157:H7.

Material and methods. The device sensitivity has been calibrated for colony forming units (CFU) from 10 to 800 yielding an error less than 11%.

Results. A correlation between optical response and the instantaneous number of bacteria was achieved. The optical output signal was 0.016 (\pm 0.001) dB per hour per bacteria. In all cases detection started after 270 ± 4 minutes, 5-10 times faster than conventional bacteriological techniques.

Discussion. The results the proposals here presented, can contribute to the real solution of pathogens monitoring and detection, solving many within several problems of Public Health.

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Key words: Optical-fibre sensor, evanescent field, bacteriological detection, *Escherichia coli* O157:H7.

RESUMEN.

Sistema de detección en línea de *Escherichia coli* O157: H7: Un nuevo apoyo para la Salud Pública.

Introducción. Motivado por los casos innumerables de infecciones de diarrea que frecuentemente ocurren, en los países en vías de desarrollo, se calibró un biosensor a fibra óptica con intensidad modulada usando como ejemplar *Escherichia coli* O157: H7.

Material y métodos. La sensibilidad del dispositivo se ha calibrado para las unidades formadoras de colonia (CFU) de 10 a 800 rindiendo un error más pequeño que 11%.

Resultados. Se obtuvo una correlación entre la

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respuesta óptica y el número instantáneo de bacterias. El signo del rendimiento óptico fue 0.016 (± 0.001) dB por hora por bacteria. En todo los casos la detección empezó después de 270 ± 4 minutos, 5-10 veces más rápido que las técnicas bacteriológicas convencionales.

Discusión. Los resultados obtenidos pueden contribuir al problema de monitoreo de patógenos que representen problemas en el campo de la salud pública. (*Rev Biomed 2000; 11:263-270*)

Palabras clave: Sensor a fibra óptica, campo evanescente, detección bacteriana, *Escherichia coli* O157:H7.

INTRODUCTION.

The greatest drawback for pathogen-detection techniques is the lack of reliable and sensitive means for measuring their presence in real time. Evanescent-wave-coupling sensor technology is a suitable technique for microorganism measurement in its natural form that may be applied to the biosensors development taking advantage of the current understanding of the whole cell architecture of the microbial (1-3).

Enterohemorrhagic *Escherichia coli* O157:H7 is an emerging pathogen, which is the cause of foodborne illness, the major cause of serious outbreaks and sporadic cases of hemorrhagic colitis and hemolytic-uremic syndrome (4-6). The major challenge of induced diarrhoeal disease is on children under the age of 10 years and elderly people, living in less-developed countries of the world in which bacterial diarrhoeal diseases remain a significant public health problem. *E. coli* O157:H7 is the cause of acute and persistent diarrhoeal disease and ongoing morbidity that has as contributors the poor nutrition (7).

The concepts of Public Health, attempts for organized effort of guidelines, objectifying within others assertives the control of transmissible infections, with medical services with diagnosis in short space of time, ally at a level sets adapted for

the health conservation. It should be observed that the prevention accomplishments in the Public Health, important of the past, sometime, they took years, decades, and even generations for they be they have concrete results. However, several environmental dangers are far away from immediate solution. The subjects of atmosphere control and health became interchangeable conceptually, and the microbiologic control became strategic, in way to allow maintainable integration in the specific manners of private or public prevention (8-10).

MATERIALS AND METHODS.

A fiberoptic biological sensor has been designed in our laboratory and was described elsewhere (11,12). In this work, using the same measurement principle, the sensor has been employed to quantify *E. coli* O157:H7. We have chosen *E. coli* O157:H7 as a prototype for the sensor development, because it is an emerging pathogen of worldwide public health importance, responsible for expressive outbreaks and by the fact that few pathogens can routinely cause such striking clinical syndromes in different organ systems as can *E. coli* O157:H7. By monitoring its presence it will be possible to diagnose and detect outbreaks.

The biological and medical areas such as living cells and bacteria detection remain a challenging technique. The advantages of fiberoptic sensing are well known and have been reported in the literature (13,14). In the last few years fiberoptic sensors have been increasingly employed in biological sensing because of their electrical isolation, electromagnetic-interference immunity, compactness, lightweight, sensitivity, in-line implementation by means of evanescent-field coupling, potential low cost and biological compatibility. The optical sensor has revealed to present a faster response than conventional techniques of clinical laboratory analysis. One of the major advantages of using the coupling with the guided lightwave evanescent field is the

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development of in-line fibreoptic devices. The fibre is not interrupted and the light is not removed from the fibre. The signal processing takes place inside the fibreoptic environment in a processes known as intrinsic sensor.

The sensing mechanism of the biosensor described here relies upon the attenuation of a guided lightwave by means of evanescent-field coupling that takes place at the fibre optic sensor. As the bacteria grow in the neighbourhood of the sensor, the guided lightwave is changed in intensity. The guided evanescent field of the lightwave penetrates beyond the core surface and exponentially decreases in its magnitude an order of a wavelength away from the core/clad reflecting interface. The biosensor described here may be envisaged as comprised by three parts: The optical circuitry, the evanescent probe-fibre and the biological culture medium. The probe-fibre was put over the culture medium in which the bacteria grows, selectively. All experiments described in this paper were performed using *Escherichia coli* O157:H7 CDC EDL-933 (ATCC 43894), supplied by the Centre for Disease Control and Prevention (CDC/USA).

The fig. 1 shows a schematic drawing of the biosensor set-up. The optical source is a 3-mW CW GaAlAs laser with graded-index multimode fibre pigtail, emitting at 840 nm. The output fibre was spliced with a bi-directional optical fibre coupler.

Half of the emitted optical power propagates over the probe-fibre (sensing element). The light modulated by the growing bacteria exits the fibre and is detected by the photodiode PD_2 providing the electrical signal $Sample_{in}$. The other half of the optical power is detected by the photodiode PD_1 from which an electrical reference signal Ref_{in} is obtained. Both electrical signals are amplified and measured by a two-channel calibrated optical power meter (Graseby Optronics). An A/D board controlled by the LabVIEW software (National Instruments Corporation) digitises both output signals from the optical power meter ($Sample_{out}$ and Ref_{out}) by means of its IEEE-488 interface. The A/D board collects 800 points per minute of both $Sample_{out}$ and Ref_{out} recording and storing the respective averages for a period of 24 hours.

For exposition of the evanescent lightwave field of the optical fibre, 20 cm of a graded-index multimode optical fibre (62.5/125 μm) was clad-stripped by chemical etching. The etching was performed by means of hydrofluoric acid solution (38%) during 11 minutes in order to leave between 0.5 to μm of clad over the core of the fibre. After this time, the chemical reaction was stopped by immersion in deionised water and then in phosphate-buffered saline (PBS) with pH 7.4 for 15 minutes in order to remove all remains of water from the probe. The exact etching time was determined by monitoring diameters in a previous

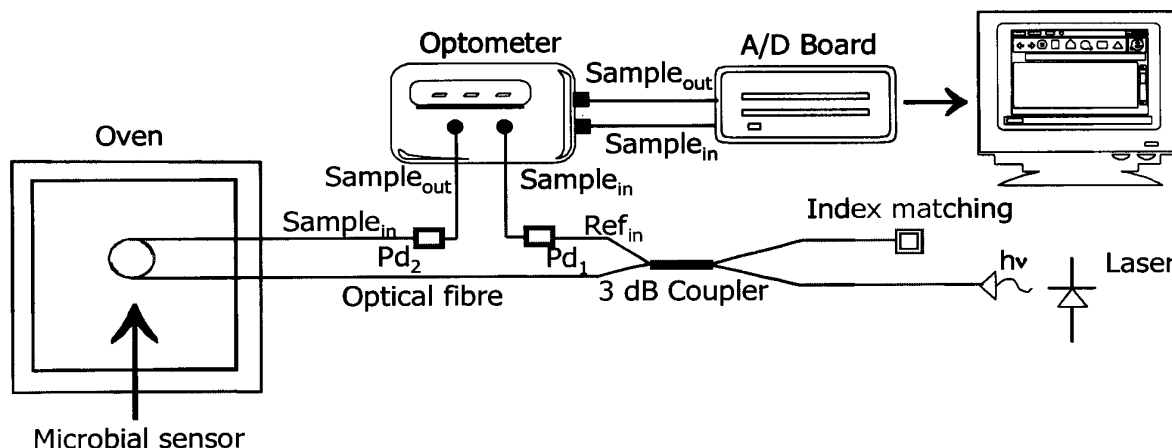


Figure 1.- Optical set-up of the biosensor.

experience using a calibrated optical microscope. This method has been described in detail (13). After the chemical etching the fibre was wound into a single loop, with a total sensitive surface area of approximately 40.0 mm², in which the evanescent field can be accessed. The *E. coli* O157:H7 growth, the selective culture medium employed was MacConkey Sorbitol Agar (SMAC) from Difco Laboratories (São Paulo/Brasil - Difco 0079-17-7) at an incubation temperature of 35°C. After optical measurements with the biosensor, the bacteria underwent microbiological identity tests in order to ensure that only that specific microorganism had grown on each Petri plate. The identity tests were made in accordance with Farmer & Davis (15).

In order to calibrate the biosensor for its sensitivity, several tests were performed using different initial number of bacteria (N_0). *E. coli* O157:H7, available lyophilised in ampoules, was restored with 1.0 ml of PBS, pH 7.4, inoculated in several Petri plates with SMAC and incubated for 35°C for 24 hours. At the end of this period, the purity of the material was checked and the Petri plates were stored at 4°C, for further dilution. For obtaining a dilution with $N_0=10, 20, 30, 40, 50, 60, 70$ and 80 microorganisms, it was used 100 l of PBS, pH 7.4 whereas for $N_0 = 90, 100, 200, 400$ and 800 the volume used was 500 l of PBS, pH 7.4. For each sample the cells were counted using the Coulter Counter (Beckman), which allows a accuracy of $\pm 1\%$. Finally, the probe was inserted into the Petri plates with the culture as described above, the dilution with a known number of microorganisms was poured onto the sensitive area of the probe and the hardware and software were started.

In all cases the SMAC was supplemented with glycerol at 0.2% which allows the maintenance of the residual humidity and provides a better interaction between the microorganisms and the sensitive area. This was done in order to avoid the culture going dry during the tests and thus inserting another variable into the process.

RESULTS.

For the biosensor sensitivity characterization, several measurements were carried out, each one during a 24-hour interval (1440 minutes) employing thirteen different values of initial numbers of bacteria, N_0 . These values varied from 10 to 800 *E. coli* O157:H7 bacteria samples.

It was chosen aleatory the graph of $n = 800$ for being the largest bacterial concentration used in the tests and, besides, explicit characteristically the three detection phases. Fig. 2 shows the plot of the temporal optical response $I_{out}(t)$ (in arbitrary units) of the measurement ($N_0 = 800$). The results of the optical measurements were reproducible. The reproducibility is also observed in all measurements corresponding to different N_0 . It should also be observed the curves closely match between themselves. The plots displayed in Fig. 2 show three different phases of $I_{out}(t)$ for any used N_0 (Lag phase, Log phase and Stationary phase). In the first phase, it may be observed a DC level $I_{out}(t)=I_{LAG}$ with an almost similar time range. The I_{LAG} level is assigned to the biosensor response in which the *E. coli* O157:H7 remains in its lag phase during Δt_{LAG} time delay.

Fig. 3 shows a plot of Δt_{LAG} against N_0 for all measurements. The linear relationship with an almost null angular coefficient was fitted with an average of 270 ± 4 minutes or approximately 4.5 hours, meaning a repeatability of $\sim 1.5\%$. Δt_{LAG} may be attributed to the time range that *E. coli* O157:H7 spent in its LAG phase despite their initial number N_0 .

The optical attenuation ΔI_{out} (in dB) for each N_0 it means the difference between the time width Δt_{LOG} (in hours) of the log phase for each N_0 . The time derivative β_{LOG} of $I_{out}(t)$ in the log phase varies with N_0 and may be calculated from Eq. 1:

$$\beta_{LOG}(N_0) \equiv \frac{\Delta I_{out}}{\Delta t_{LOG}} \quad (1) \quad \beta_{LOG}(N_0) \equiv \frac{\Delta I_{out}}{\Delta t_{LOG}}$$

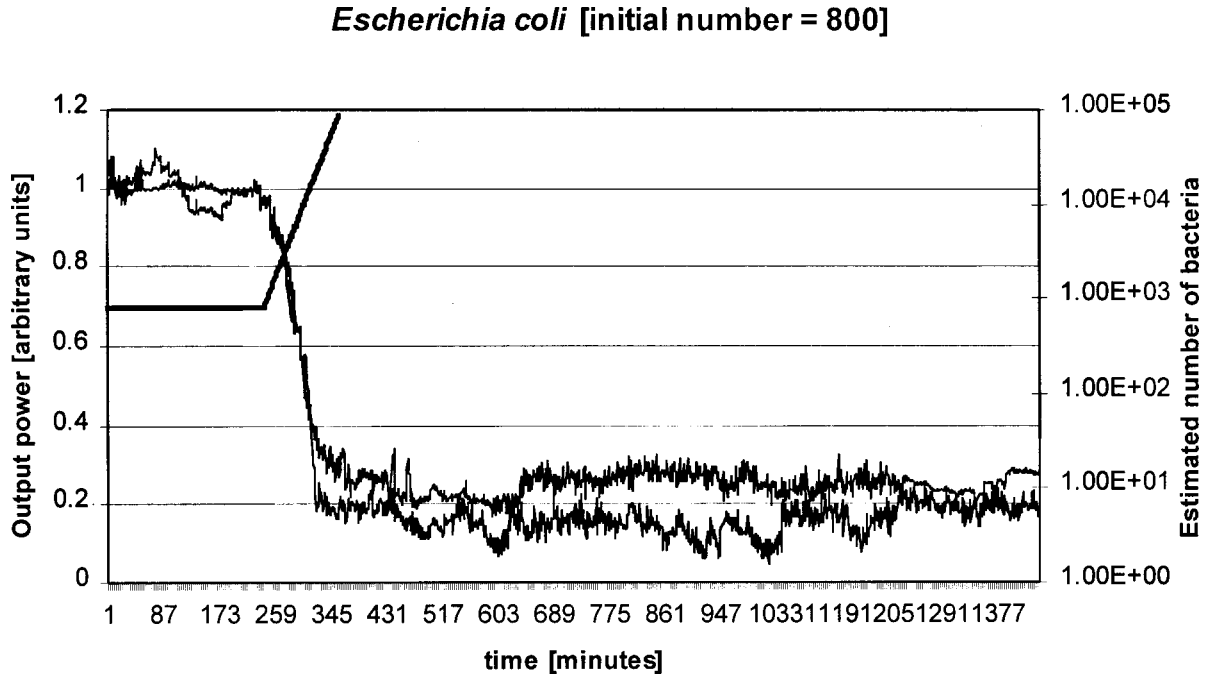


Figure 2.- Optical response $I_{out}(t)$ of the biosensor parameterised by the initial number $N_0 = 800$ of *E. coli* O157:H7.

Fig. 4 shows on the left vertical axis, the optical attenuation ΔI_{out} occurred during the log phase (expressed in dB) against the N_0 (horizontal axis). Fig. 4 also shows on the right vertical axis, the time range Δt_{LOG} during which the *E. coli* O157:H7 spent in the log phase (expressed in minutes) against the N_0 (horizontal axis).

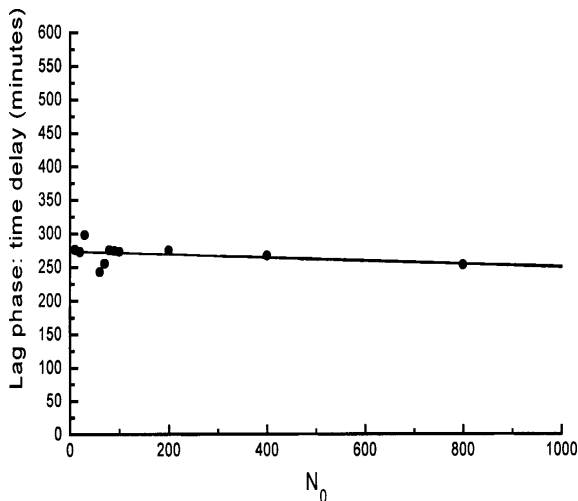


Figure 3.- Time delay in the LAG phase Δt_{LAG} (minutes) against the initial number N_0 of *E. coli* O157:H7.

Although ΔI_{out} and Δt_{LOG} are different quantities (with different dimensionality), a direct comparison between both should be carried out with careful. But from a qualitative point of view, Fig. 4 clearly shows that both ΔI_{out} and Δt_{LOG} follows a complementary rule. The ΔI_{out} exponentially increases with N_0 until saturation. On the other hand, the Δt_{LOG} exponentially decreases until an almost null value.

The angular coefficient was calculated to be $\Delta\beta_{LOG}/\Delta N_0 = (0.016 \pm 0.001)$ (dB/hour)/bacteria meaning that for each *E. coli* O157:H7 bacterium inoculated upon the Petri plate, the speed of the biosensor response at the log phase increases by 0.016 dB/hour. For $N_0 = 800$ the angular coefficient $\beta_{LOG}(800) \sim 6$ dB/h. Therefore, it is possible to tell from the output signal, the initial number of bacteria by measuring the angular coefficient of the log phase. The CFU is directly related to the degree of contamination of the sample, when applying the system *in vivo*.

DISCUSSION.

The biosensor response relies on the interaction between the lightwave and the bacteria causing the optical attenuation at the probe-fibre. The optical interaction raises from the evanescent-field coupling since there is a physical contact between the fibre and the bacteria as shown in Fig. 2. The physical contact between the SiO_2 (probe-fibre) and the whole *E. coli* O157:H7 occurs because the bacteria is allowed to grow around the fiber-probe. This mechanism greatly simplifies the construction of a biosensor when compared with other processes, for instance the silanization technique (16). This technique consists of a complex immune procedure by which means antibodies from microbiological species are covalently linked to the SiO_2 of the fiber thus forming a tightly structure capable of coupling the lightwave travelling inside the fiber.

The bacteria that touched the fiber during the inoculation, together with those that happen

to grow over and around the fiber are within the evanescent field and are sensed and monitored. However, since the probe-fibre was rested upon the surface of the biological medium prior to the optical measurements, the lightweight probe-fibre was not completely buried inside the gel-like culture medium because of the superficial tension and the nutrients in excess slowly slide down from the top of the probe-fibre. Also due to the surface tension, only a thin film of culture medium will remain touching the fibre. Therefore, the bacteria that happen to be isolated in the probe-fibre will grow and reproduce until the exhaustion of this limited amount of nutrients. The calibration of the biosensor sensitivity with N_0 as shown by Fig. 4, features a linear dependence of $\beta_{\text{LOG}} (= \Delta I_{\text{out}} / \Delta t_{\text{LOG}})$ from $N_0=10$ until $N_0=400$. For $N_0=800$ it was observed a deviation from the linear dependence which suggests a possible saturation of the biosensor response.

In order to increase the sensitivity and the

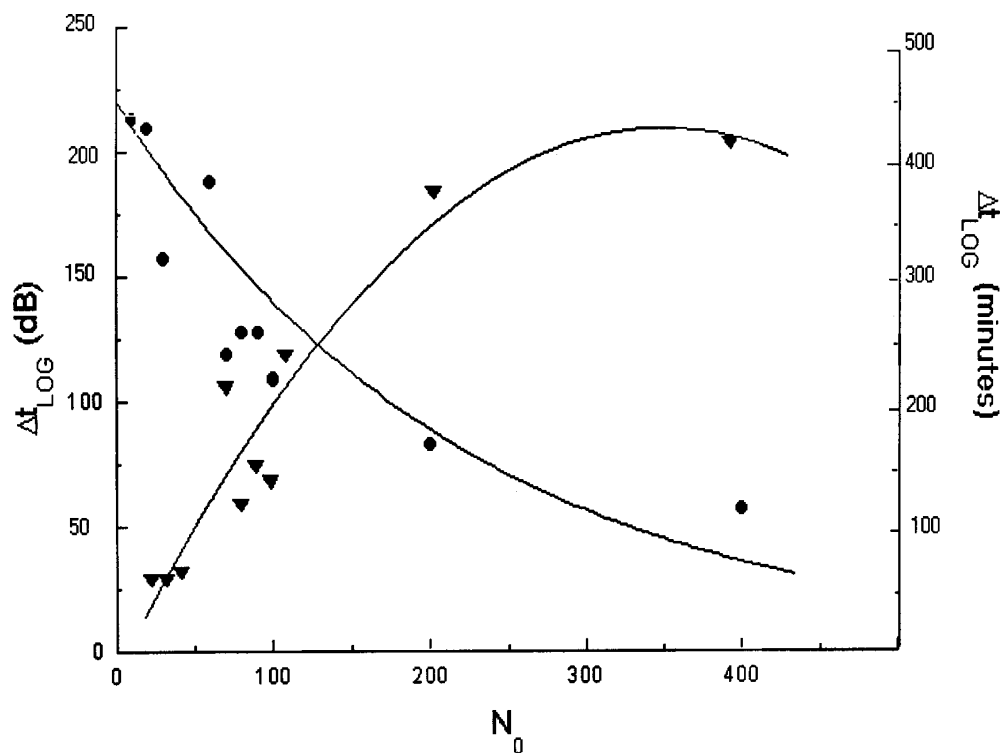


Figure 4.- Attenuation ΔI_{out} at the LOG phase (dB) on the left vertical axis and the Δt_{LOG} (minutes) on the right vertical axis both plotted against the initial number of bacteria N_0 in the horizontal axis.

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time derivative (speed at the log phase) of the biosensor in its presently basic configuration, some simple improvements are suggested: *a*) Optimisation of the wavelength for a better sensitivity. For instance, by using a longer wavelength we would have a larger sensitivity area because the evanescent field would also be larger. By testing the sensor with a modulated light source, several wavelengths could be tested; *b*) The use of a longer probe-fibre (the actual measures 20 cm) will absorb more light and therefore will present a higher sensitivity for the same bacteria concentration; *c*) Since it is possible to detect only one microorganism at a time, one approach to overcome this limitation would be the miniaturisation of the probe fibre such that an array of them, each one specific for different microorganisms, could be wavelength division multiplexing (WDM).

When being the illnesses of transitory or permanent incidence, its repercussion can be evidenced by political assistances, even so when such illnesses are of infect-contagious nature, consequently the damages in the population have, at times expressive, larger transcendence. The control of the transmissible diseases bases on interventions that, acting on one or more well-known links of the epidemic chain of transmission, be capable to come to interrupt it. However, the man's interaction with the environment is very complex, involving unknown factors or that can have been modifying him in the moment in that the action is unchained. Like this being, the intervention methods tend to be turn better or substituted, in the measure in that new knowledge are contributed, be for scientific discoveries (therapeutics, physio-pathogenic or epidemic), be for the systematic observation of the behaviour, of the prevention procedures and established control. The evolution of these knowledge contributes, also, for the modification of concepts and organizational forms of the services of health, in the continuous search of its improve (17,18).

An evanescent-field and intensity-modulated

fibreoptic sensor for detection and monitoring of *E. coli* O157:H7 has been described and calibrated. This bacteria has been successfully detected and quantified in its natural form, 5-10 times faster than conventional bacteriological techniques. With this system it is possible to tell which bacteria has been inoculated and its concentration. The development of this highly sensitive and selective probe for real time pathogen detection has improved biological sensing. With minor modifications the method can be used to test for food contamination as well as for clinical essays and environmental monitoring. We believed that the results the proposals here presented, can contribute to the real solution of pathogens monitoring and detection, solving many within several problems of Public Health.

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