Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytical Biochemistry 337 (2005) 195–202
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Continuous-flow/stopped-flow system using an immunobiosensor for quantification of human serum IgG antibodies to *Helicobacter pylori*

Germán A. Messina, Angel A.J. Torriero, Irma E. De Vito, Roberto A. Olsina, Julio Raba*

Department of Chemistry, National University of San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina

Received 7 June 2004
Available online 25 December 2004

Abstract

Conventional methods, such as gastric biopsy, enzyme-linked immunosorbent assay (ELISA), culture, require a long time for the determination of *Helicobacter pylori* infections. This study reports an amperometric immunoreactor for rapid and sensitive quantification of human serum immunoglobulin G (IgG) antibodies to *H. pylori.* Antibodies in the serum sample are allowed to react immunologically with the purified *H. pylori* antigens that are immobilized on a rotating disk. The bound antibodies are quantified by horseradish peroxidase (HRP) enzyme-labeled second antibodies specific to human IgG. HRP in the presence of hydrogen peroxide catalyzes the oxidation of hydroquinone to *p*-benzoquinone. The electrochemical reduction back to hydroquinone is detected on a glassy carbon electrode surface at −0.15 V. The electrochemical detection can be done within 1 min, and the analysis time does not exceed 30 min. The calculated detection limits for amperometric detection and the ELISA procedure are 0.6 and 1.9 U ml^{-1}, respectively. The amperometric immunoreactors showed higher sensitivity and lower time consumed than did the standard spectrophotometric detection ELISA method. It can also be used for rapid analysis in conventional and field conditions in biological, physiological, and analytical practices.

Keywords: ELISA; *Helicobacter pylori*; Amperometric immunoreactor; Horseradish peroxidase; Hydroquinone; FIA

Helicobacter pylori is a gram-negative, spiral-shaped bacillus that, in close contact with the gastric epithelium, can cause peptic ulcers and chronic gastritis [1,2]. *H. pylori* infection causes nearly all ulcer diseases that are not brought about by the use of certain medications such as nonsteroidal antiinflammatory drugs [3]. Evidence linking chronic *H. pylori* infection and gastric cancer has also been demonstrated by epidemiological and pathological studies [4,5].

The discovery of *H. pylori* as the causative organism for most of the gastritis and peptic diseases has revolutionized the treatment and management of these diseases. The disease can be cured by eradication of *H. pylori* through the triple therapies based on a proton pump inhibitor with two antibiotics (clarithromycin and metronidazole or amoxicillin) [6]. To prevent the indiscriminate use of multiple antibiotics, an accurate diagnosis for the presence of *H. pylori* infection becomes crucial. Current methods for diagnosing *H. pylori* infection can be divided into invasive and noninvasive (or minimally invasive) [7]. The invasive method uses endoscopy to obtain a biopsy sample of the stomach lining, culturing, and histological examination of the biopsy sample to demonstrate the presence of *H. pylori.* Alternatively, a positive urease test in the biopsy sample indicates the presence of *H. pylori* because *H. pylori* contain large quantities of the enzyme urease [8,9]. The noninvasive tests include the urea breath test and serological tests.

* Corresponding author. Fax: +54 2652 43 0224.
E-mail address: jraba@unsl.edu.ar (J. Raba).

0003-2697/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2004.07.027

Some noninvasive tests are based on serological procedures that detect immunoglobulin G (IgG)[1] against *H. pylori* in human serum. Circulating anti-*H. pylori* IgG antibody has proved to be of considerable value in the diagnosis of active infection due to the reliable correlation between the presence of the antibody and gastric mucosal colonization [10,11]. Common serum IgG measurements are carried out using enzyme-linked immunosorbent assay (ELISA) [12]. Although ELISA is a popular assay in clinical examination, the relatively poor limit of detection with absorbance spectrophotometry is one cause of its lack of sensitivity [13]. In all instances, the immobilized antigens are discarded after only one use.

Heterogeneous enzyme immunoassays, coupled with flow injection (FI) system and amperometric detection, represent a powerful analytical tool for the determination of low levels of many analytes such as antibodies, hormones, drugs, tumor markers, and viruses [14]. Amperometric detection offers good to excellent sensitivity combined with simple and low-cost instrumentation [15,16]. One approach used in such techniques is to employ an enzyme label that generates an electrochemically active product [17–20].

In this article, we establish an amperometric immunoreactor with rotation incorporated into an FI analytical system for rapid and sensitive quantification of human serum IgG antibodies to *H. pylori*, based on the use of purified *H. pylori* antigens that are immobilized on a rotating disk. Antibodies in the serum sample are allowed to react immunologically with the antigens, and the bound antibodies are quantified by horseradish peroxidase (HRP) enzyme-labeled second antibodies specific to human IgG, using hydroquinone (H_2Q) as enzymatic mediators. HRP in the presence of hydrogen peroxide (H_2O_2) catalyzes the oxidation of H_2Q to *p*-benzoquinone (Q) [21]. The electrochemical reduction back to H_2Q is detected on a glassy carbon electrode (GCE) surface at −0.15 V. The response current obtained from the product of enzymatic reaction is proportional to the activity of the enzyme and, consequently, to the amount of antibodies bound to the surface of the immunosensor of interest.

Materials and methods

Reagents and solutions

All reagents used were of analytical reagent grade. HRP enzyme-labeled second antibodies specific to human IgG were purchased from Sigma Chemical (St. Louis, MO, USA). Glutaraldehyde (25% aqueous solution) and H_2O_2 were purchased from Merck (Darmstadt, Germany). 3-Aminopropyl-modified controlled pore glass, 1400 Å mean pore diameter and 24 $m^2\,mg^{-1}$ surface area, was obtained from Electro Nucleonics (Fairfield, NJ, USA) and contained 48.2 $\mu mol\,g^{-1}$ of amino groups. H_2Q was purchased from Sigma Chemical, and all other reagents employed were of analytical grade and used without further purifications. Aqueous solutions were prepared using purified water from a Milli-Q system.

The ELISA test kit for the quantitative determination of *H. pylori*-specific IgG class antibodies was purchased from EQUIPAR Diagnostici (Rome, Italy) and was used in accordance with the manufacturer's instructions [22].

Flow-through reactor/detector unit

The main body of the cell was made of Plexiglas Fig. 1 illustrates the design of the flow-through chamber containing the rotating immunoreactor and the detector system. GCE is on the top of the rotating reactor. The rotating reactor is a disk of Teflon in which a miniature magnetic stirring bar (Teflon-coated Micro Stir bar from Markson Science, Phoenix, AZ, USA) has been embedded. Typically, a reactor disk carried 1.4 mg of controlled pore glass on its surface. Rotation of the lower reactor was effected with a laboratory magnetic stirrer (Metrohm E649 from Metrohm AG, Herisau, Switzerland) and controlled with a variable transformer with an output between 0 and 250 V and maximum amperage of 7.5 A (Waritrans, Argentina). Amperometric detection was performed using the BAS CV27, and the BAS 100 B (electrochemical analyzer from Bioanalytical Systems, West Lafayette, IN, USA) was used for cyclic voltammetric analysis. The potential applied to the GCE for the functional group detection was −0.15 V versus Ag/AgCl, 3.0 M NaCl reference electrode BAS RE-6, and a Pt wire counter electrode. At this potential, a catalytic current was well established. A pump (Gilson Minipuls 3 peristaltic pump, Gilson Electronics, Middleton, WI, USA) was used for pumping, introducing the sample, and stopping the flow Fig. 2 illustrates schematically the components of the single-line continuous-flow setup. The pump tubing was Tygon (Fisher Accu-Rated, 1.0 mm i.d., Fisher Scientific, Pittsburgh, PA, USA), and the remaining tubing used was Teflon (1.0 mm i.d. from Cole–Parmer, Chicago, IL, USA).

All pH measurements were made with an Orion Expandable Ion Analyzer (model EA 940, Orion Research, Cambridge, MA, USA) equipped with a glass combination electrode (Orion Research). The absorbancies were detected by Bio-Rad Benchmark microplate readers (Japan) and a Beckman DU 520 general UV/

[1] *Abbreviations used:* IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay; FI, flow injection; HRP, horseradish peroxidase; H_2Q, hydroquinone; H_2O_2, hydrogen peroxide; Q, *p*-benzoquinone; GCE, glassy carbon electrode; PBS, phosphate-buffered saline; APCPG, 3-aminopropyl-modified controlled pore glass; FIA, flow injection analysis.

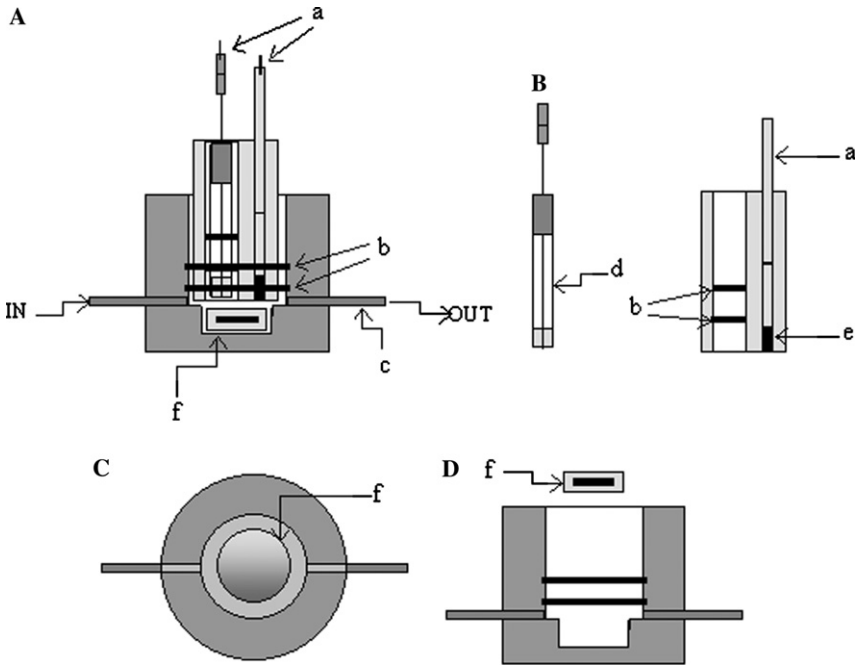


Fig. 1. Schematic representation of components in the bioreactor flow cell: (A) assembled reactor, (B) upper cell body, (C) top view of lower cell body, and (D) lower cell body. a, Electrical connection; b, O-ring; c, auxiliary electrode; d, Ag/AgCl, 3 M NaCl reference electrode BAS RE-6; e, GCE; f, rotating bioreactor (with immobilized HRP).

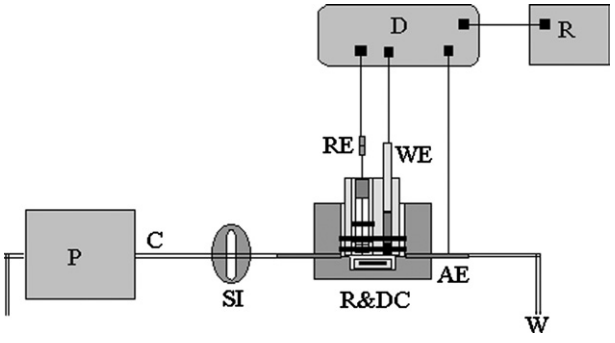


Fig. 2. Block diagram of the continuous-flow system and detection arrangement. P, pump (Gilson Minipuls 3 peristaltic pump); C, carrier buffer line; SI, sample injection; W, waste line; R & DC, reactor and detector cell; WE, GCE; RE, reference electrode (Ag/AgCl, 3 M NaCl); AE, auxiliary electrode (platinum); D, potentiostat/detection unit (BAS CV27); R, recorder (model 9176, Varian Techtron, Springuale, Australia).

Vis spectrophotometer (Fullerton, CA, USA). The *H. pylori* antigens were sonicated by a Sonics Vibra Cell ultrasonic processor (Sonics & Materials, Newtown, CT, USA).

H. pylori-specific IgG antibody immunoassay

A series of standards that covered the clinically relevant range (0–100 U $m1^{-1}$) was supplied with the ELISA test kit. A standard curve for the spectrophotometric procedure was produced by following the manufacturer's protocol. Concentrations of *H. pylori*-specific IgG antibody were detected spectrophotometrically by measuring absorbance changes at 450 nm.

Preparation of the H. pylori antigens

The antigens was prepared from a sonicate *H. pylori* culture strain. The *H. pylori* antigens were grown on blood agar plates at 37 °C for 3 days and then harvested, washed, and resuspended in 0.01 M phosphate-buffered saline (PBS, pH 7.2). This preparation was subjected to sonication. The sonic amplitude level was set at 20, and the machine was operated using four cycles of 60 s regulated alternatively. The sonicated preparation was centrifuged at 1000*g* for 10 min, and the supernatant was stored in the 0.01 M PBS (pH 7.2), at −20 °C between uses.

H. pylori antigens immobilization

The rotating disk reactor (bottom part) was prepared by immobilizing *H. pylori* antigens on 3-aminopropyl-modified controlled pore glass (APCPG). The APCPG, smoothly spread on one side of a double-coated tape affixed to the disk surface, was allowed to react with an aqueous solution of 5% (w/w) glutaraldehyde at pH 10.0 (0.20 M carbonate) for 2 h at room temperature. After washing with purified water and 0.10 M phosphate buffer (pH 7.0), 0.5 ml of antigens preparation (100 μg ml^{-1} 0.01 M PBS, pH 7.2) was coupled to the residual aldehyde groups overnight at 5 °C. The immobilized antigens preparation was finally washed with

phosphate buffer (pH 7.0) and stored in the same buffer at 5 °C between uses. The immobilized *H. pylori* antigen preparations were perfectly stable for at least 1 month.

Amperometric analysis of H. pylori-specific IgG in human serum samples

This method was applied in the determination of IgG antibodies to *H. pylori* in 19 human serum samples. The unspecific binding was blocked by 10-min treatment at 37 °C with 3% descremate milk in a 0.01-M PBS (pH 7.2). The serum samples were first diluted 100-fold with 0.01 M PBS (pH 7.2) and then injected into the PBS carrier stream at a flow rate of 1 ml min^{-1} and incubated 10 min at 37 °C. The immunoreactor was washed with 0.01 M PBS (pH 7.2) at a flow rate of 1 ml min^{-1}. The anti-human IgG–peroxidase conjugate (dilution of 1/2000 in 0.01 M PBS, pH 7.2) was then injected into the 0.01 M PBS (pH 7.2) carrier stream and incubated 10 min at 37 °C.

The immunoreactor was then washed free of any traces of unbound enzyme conjugate with 0.01 M PBS (pH 7.2). Finally, the substrate solution (100 µl of a 0.1-M phosphate–citrate buffer, pH 5.05, containing 1×10^{-3} mol L^{-1} H_2O_2 and 1×10^{-3} mol L^{-1} H_2Q) was injected into the carrier stream, and the enzymatic product was detected by amperometric FI analysis. For the next analysis, the immunoreactor was conditioned by injection of desorption buffer (0.1 M glycine–HCl, pH 2) for 2 min and then washed with 0.01 M in PBS (pH 7.2).

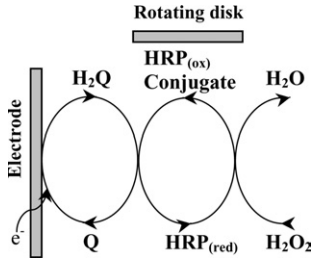
A standard curve for the amperometric procedure was produced by following our protocol with a series of standards that covered the clinically relevant range (0–100 U $m1^{-1}$) supplied with the ELISA test kit. Amperometric measurements were performed at −0.15 V at room temperature in a 0.1-M phosphate–citrate buffer (pH 5.05) solution containing 1×10^{-3} mol L^{-1} H_2O_2 and 1×10^{-3} mol L^{-1} H_2Q, and the resulting cathodic current was displayed on the $x - y$ recorder. When not in use, the immunoreactor was stored in 0.01 M PBS (pH 7.2) at 4 °C.

Results and discussion

Study of the enzymatic process

Reactions catalyzed by enzymes have long been used for analytical purposes in the determination of different analytes such as substrates, inhibitors, and the enzymes. The catalysis mechanism of HRP was explained in Ref. [23].

HRP in the presence of H_2O_2 catalyzes the oxidation of H_2Q to Q [21], which at a potential of −0.15 V was electrochemically reduced to H_2Q, providing a peak current related to its concentration [24]. A schematic representation of this process is shown in Scheme 1.



Scheme 1. Schematic representations of the reduction wave of the enzymatic process among hydroquinone (H_2Q), *p*-benzoquinone (Q), hydrogen peroxide (H_2O_2), and horseradish peroxidase (HRP) conjugated.

Effect of reactor rotation and continuous-flow/stopped-flow operation

The implementation of continuous-flow/stopped-flow programming and the location of two facing independent reactors (Fig. 1) permits (i) use of relatively low immunoreactant loading conditions, (ii) instantaneous operation under high initial rate conditions, (iii) easy detection of accumulated products, and (iv) reduction of apparent Michaelis–Menten constant (K'_M).

A more complete reagent homogenization is achieved [25] because the cell works as a mixing chamber by facilitating the arrival of immunoreactants at the specific antigens, the arrival of enzymatic substrate at the active sites, and the release of products from the same sites. The net result is high values of current. The main advantages of this system are its simplicity and the ease with which it can be applied to the determination of specific IgG antibodies to *H. pylori* in serum samples.

The effect of the rotation velocity was studied in a range of 60–250 rpm. A linear relationship between the electric signal and rotation velocity was observed in the range of 60–180 rpm and showed a maximum rate of response at 180 rpm [26].

If the reactor in the cell is devoid of rotation, the response is lower because diffusional reactions are too slow to be observed in the time scale of amperometric analysis (Fig. 3D). If a rotation of 180 rpm is imposed on the reactor located at the bottom of the cell (with *H. pylori* antigens immobilized and the immunocomplex with antibodies of serum samples and the enzyme conjugated), the signal is dramatically enlarged (Fig. 3).

The response current obtained from the oxidation of H_2Q in aqueous solution containing 0.1 M phosphate–citrate buffer (pH 5.05) is proportional to the activity of the enzyme conjugated and, consequently, to the amount of specific antibodies of serum samples bound to the rotating disk with *H. pylori* antigens immobilized.

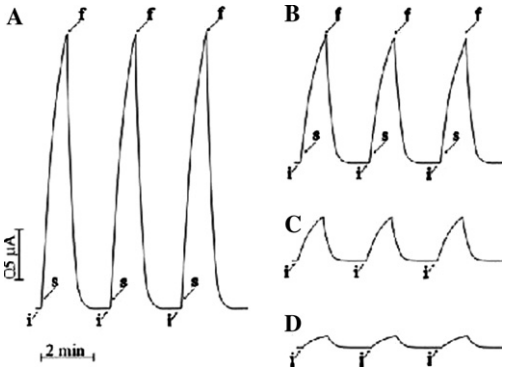


Fig. 3. Effect of reactor's rotation under continuous-flow and stopped-flow conditions: (A) stopped flow with rotation, (B) continuous flow with rotation, (C) stopped flow without rotation, and (D) continuous flow without rotation. Flow rate 1.0 ml min^{-1}, cell volume 300 μl, 0.1 M phosphate–citrate buffer (pH 5.05) solution containing 1.0×10^{-3} mol L^{-1} H_2O_2 and 1.0×10^{-3} mol L^{-1} H_2Q. The flow was stopped for 60 s during measurement.

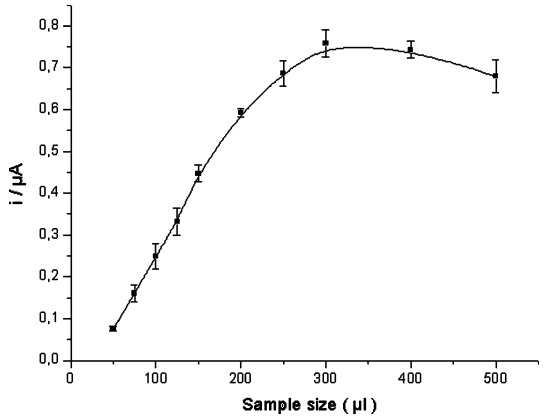


Fig. 4. Effect of sample size. The initial rate was measured under stopped-flow conditions. Each value of *i*/μA is based on five determinations. Flow rate 1.0 ml min^{-1}, reactor rotation velocity 180 rpm, 0.1 mol L^{-1} phosphate–citrate buffer (pH 5.05) solution containing 1.0×10^{-3} mol L^{-1} H_2O_2 and 1.0×10^{-3} mol L^{-1} H_2Q. The flow was stopped for 60 s during measurement.

As noted previously, rotation is expected to decrease the values of the apparent K'_M because the catalytic efficiency is increased. A K'_M value that differs substantially from that measured in homogeneous solution is not an intrinsic property of the enzyme; rather, it is an intrinsic property of the system. This constant characterizes the reactor, not the enzyme itself. It is a measure of the substrate concentration range over which the reactor response is linear [27].

Effect of cell volume and sample size

Depending on the volume of the cell in contact with the reactor, the overall process becomes controlled by diffusion (large volumes) or by the chemical kinetics of the immune and enzyme-catalyzed reactions (small volumes). The cell volume was changed from 300 μl to 1 ml by removing the O-rings between the upper and lower halves of the cell. The rate of response, as expected, decreased linearly with an increase in cell volume due to the dilution effect favored by rotation and the fact that the measured current is directly proportional to bulk concentration. The smallest cell volume of 300 μl was adopted for further studies. The rate of response increased linearly with sample size up to 200 μl in a cell with a volume of 300 μl. For convenience, a sample size of 200 μl was used to evaluate other parameters. Sensitivity is nearly tripled in the range between 50 and 200 μl (Fig. 4).

Optimal conditions for the determination of the enzymatic products

The rates of enzymatic response under stopped-flow conditions were studied in the pH range of 3–8 and show a maximum value of activity at pH 5.05 (Fig. 5). The pH value used was 5.05 in 0.1 M phosphate–citrate buffer. The effect of varying H_2O_2 concentration from 7.0×10^{-4} to 5.0×10^{-3} mol L^{-1} for 1.0×10^{-3} mol L^{-1} H_2Q solution, and the effect of varying H_2Q concentration from 1.0×10^{-4} to 3.6×10^{-2} mol L^{-1} for 1.0×10^{-3} mol L^{-1} H_2O_2 solution, on the bioreactor response was evaluated. The optimal H_2O_2 and H_2Q concentrations found were 1.0×10^{-3} and 1.0×10^{-3} mol L^{-1}, respectively. Those concentrations were then used.

An additional parameter that would affect the assay was the incubation temperature. Different incubation temperatures, ranging from 25 to 37 °C, have been reported in the literature [28,29]. As is well known, an optimal temperature of immunoreaction would be 37 °C. At this temperature, however, a long incubation time would decrease the activity of antigen, antibody,

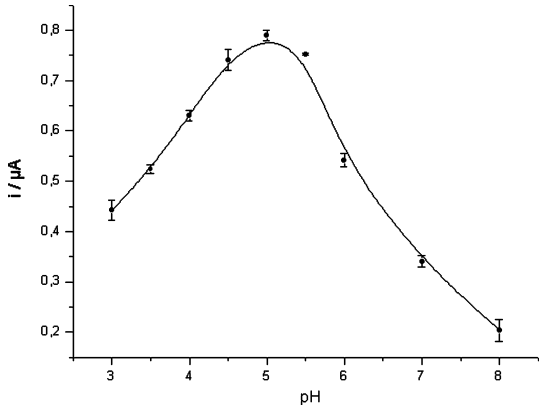


Fig. 5. Effect of pH on the rate of enzymatic response. Flow rate 1.0 ml min^{-1}, cell volume 300 μl, 0.1 M phosphate–citrate buffer (pH 5.05) solution containing 1.0×10^{-3} mol L^{-1} H_2O_2 and 1.0×10^{-3} mol L^{-1} H_2Q. The flow was stopped for 60 s during measurement.

and enzyme, leading to the deterioration of response signals and a shorter lifetime of the immunosensor. Enzyme exhibits the best activity over the temperature range of 20–25 °C; a higher temperature would be harmful to its activity [30].

Quantitative test for the detection of H. pylori-specific IgG antibody

Under the selected conditions described above, the amperometric response of the enzymatic product is proportional to the concentration of *H. pylori*-specific IgG antibody in the serum.

A linear calibration curve for the detection of *H. pyroli*-specific IgG antibody in serum was produced over the range of 0–100 U ml^{-1}, as shown in Fig. 6. The linear regression equation was $i = 0.033\ CHp + 0.131$ with the linear relation coefficient $r = 0.998$. The coefficient of variation (CV) for the determination of 20 U ml^{-1} *H. pylori*-specific antibodies was 2.9% (six replicates). The ELISA procedure was also carried out as described previously, absorbance changes were plotted against the corresponding *H. pylori*-specific IgG antibody concentration, and a calibration curve was constructed. The linear regression equation was $A = 0.028\ CHp + 0.137$ (Fig. 7) with the linear relation coefficient $r = 0.995$, and the CV for the determination of 20 U ml^{-1} *H. pylori*-specific antibodies was 4.7% (six replicates).

With the detection limit (DL) taken as the concentration that gives a signal three times the standard deviation of the blank, the DLs for amperometric detection and the ELISA procedure were 0.6 and 1.9 U ml^{-1}, respectively. This result shows that amperometric detection was more sensitive than the spectrophotometric method.

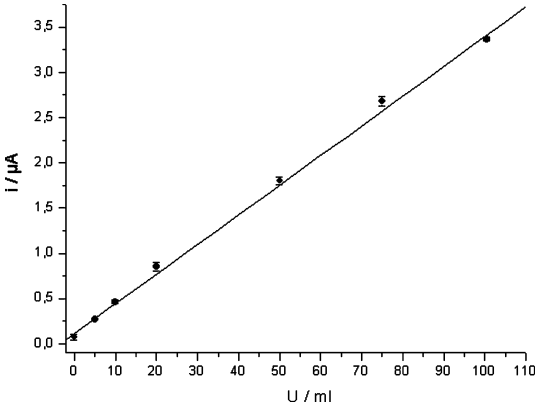


Fig. 6. Calibration curve for determination of *H. pylori*-specific IgG antibodies by amperometric FI immunoassay. Amperometric measurements were performed at −0.15 V at room temperature in a 0.1-M phosphate–citrate buffer (pH 5.05) solution containing 1.0×10^{-3} mol L^{-1} H_2O_2 and 1.0×10^{-3} mol L^{-1} H_2Q, and the resulting cathodic current was displayed on the $x - y$ recorder.

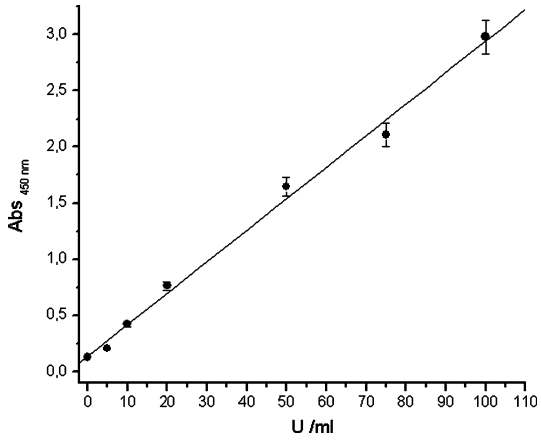


Fig. 7. Calibration curve for determination of *H. pylori*-specific IgG antibodies by ELISA.

With sensitivity defined as the slope of the regression line signal versus concentration, sensitivities for amperometric detection and the ELISA procedure were 0.033 μA/U ml^{-1} and 0.028 Abs/U ml^{-1}, respectively. The corresponding analytical performances are summarized in Table 1.

The precision of the amperometric assay was checked with control serum at 20, 50, and 100 U ml^{-1} *H. pylori*-specific antibody concentrations. The within-assay precision was tested with five measurements in the same run for each serum. These series of analyses were repeated for 3 consecutive days to estimate the between-assay precision. The results obtained are presented in Table 2. The *H. pylori* assay showed good precision; the CV within-assay values were below 3%, and the between-assay values were below 5%.

Table 1
Analytical performances of *H. pylori* assays with amperometric and photometric detection

	Amperometry	Photometry
Sensitivity	0.033 μA/U ml^{-1}	0.028 Abs/U ml^{-1}
DL	0.6 U ml^{-1}	1.9 U ml^{-1}
CV[a]	2.9%	4.7%

[a] 20 U ml^{-1} *H. pylori*-specific antibodies ($n = 6$).

Table 2
Within-assay precision (five measurements in the same run for each control serum) and between-assay precision (five measurements for each control serum, repeated for 3 consecutive days)

Control sera[a] (U ml^{-1})	Within-assay		Between-assay	
	Mean	CV percentage	Mean	CV percentage
20	20.32	1.78	21.61	2.92
50	49.74	2.54	50.04	4.20
100	99.01	2.86	98.34	3.96

[a] U ml^{-1} *H. pylori*-specific antibodies.

The accuracy was tested with dilution and recovery tests. A dilution test was performed with 100 U ml^{-1} *H. pylori*-specific antibodies control sera with 0.01 M PBS (pH 7.2) (Fig. 8).

Reproducibility assays were made using a repetitive standard (n = 6) of 20 U ml^{-1} *H. pylori*-specific antibodies; the percentage standard error was less than 3% (Table 3). The immunoreactor was regenerated by injection of desorption buffer (0.1 M glycine–HCl, pH 2) for 2 min and then washed with 0.01 M in PBS (pH 7.2), thereby permitting our reactor to be used over 100 determinations. For the next analysis, the immunoreactor was conditioned by injection of desorption buffer (0.1 M glycine–HCl, pH 2) for 2 min and then washed with 0.01 M in PBS (pH 7.2).

Fig. 9 gives the results obtained using the two methods for five separate determinations of 19 human serum samples. The results were compared, and there was no significant difference between the methods.

The long-term stability of the immunoenzymatic system to *H. pylori*-specific IgG antibodies was studied. In this experiment, after 10 samples, a standard of 20 U ml^{-1} *H. pylori*-specific antibodies was injected to test the electrode response. The catalytic current, in practice, does not decay after 10 signs.

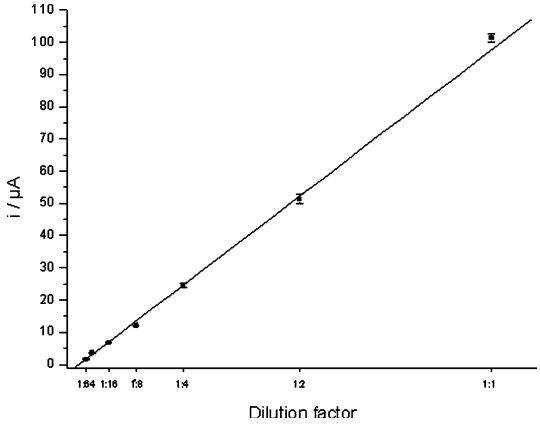


Fig. 8. Dilution test results for 100 U ml^{-1} *H. pylori*-specific antibodies. Each value of *i*/µA is based on five determinations.

Table 3
Reproducibility assays using repetitive standards (n = 6) of 20 U ml^{-1} *H. pylori*-specific antibodies

Standard of 20 U ml^{-1} *H. pylori*-specific antibodies	Proposed method (U ml^{-1})	ELISA (U ml^{-1})
1	20.11	21.43
2	19.79	19.37
3	20.64	20.47
4	20.18	19.74
5	19.53	20.92
6	20.03	21.86
Mean ± SE	20.21 ± 0.245	20.63 ± 0.393
SD	0.601	0.963

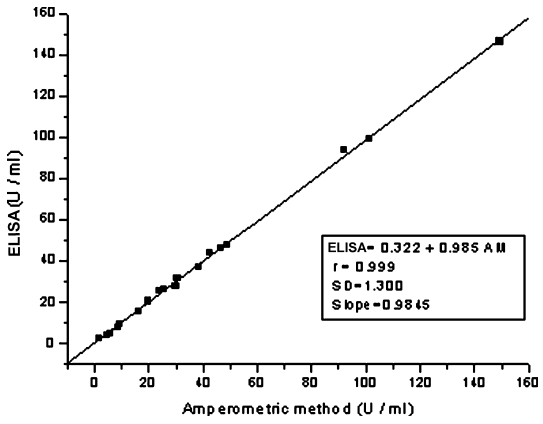


Fig. 9. Correlation between amperometric and commercial photometric assays.

Correlations with ELISA assay procedure

The amperometric system was compared with a commercial photometric system for the quantification of *H. pylori*-specific antibodies in 19 serum samples. The slopes obtained were reasonably close to 1.0, indicating a good correspondence between the two methods (Fig. 9).

Conclusions

The usefulness of the immunoreactor used for the rapid quantification of specific antibodies against *H. pylori* in human serum sample was demonstrated. In practice, the immunoreactor developed in this study is able to operate as a fast, selective, and sensitive detection unit when it is incorporated into a flow injection analysis (FIA) system. It also minimizes the waste of expensive antigens and other reagents; shows physical and chemical stability, low background current, wide working potential range, and accuracy; and does not require highly skilled technicians or expensive and dedicated equipment. The electrochemical detection can be done within 1 min, and the analysis time does not exceed 30 min. Immunosensors based on specific reactions between antibodies and antigens provide promising alternative tools for the routine clinical assay, biological, physiological, and analytical practices.

Acknowledgments

The authors acknowledge the financial support from the Universidad Nacional de San Luis and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Germán A. Messina acknowledges support

in the form of a fellowship from the Ciencia y Técnica (UNSL), and Angel A. J. Torriero acknowledges support in the form of a fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). The authors also thank Silvia Digenaro, National University of San Luis, for critical reading of the manuscript and helpful discussions. They also thank Eduardo Messina, San Luis, for the reactor's manufacture.

References

[1] B.J. Marshall, J.R. Warren, Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration, Lancet 8390 (1984) 1311–1315.
[2] A. Morris, G. Nicholson, Ingestion of *Campylobacter pylori* causes gastritis and raised fasting gastric pH, Am. J. Gastroenterol. 82 (1987) 192.
[3] M.J. Blaser, The bacteria behind ulcers, Sci. Am. 274 (1996) 104–107.
[4] J.G. Fox, P. Correa, N.S. Taylor, N. Thompson, E. Fontham, E. Janney, M. Sobhan, B. Ruiz, F. Hunter, High prevalence and persistence of cytotoxin-positive *Helicobacter pylori* strains in a population with high prevalence of atrophic gastritis, Am. J. Gastroenterol. 87 (1992) 1554–1560.
[5] C.P. Dooley, H. Cohen, P.L. Fitzgibbons, M. Bauer, M.D. Appleman, Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons, N. Engl. J. Med. 321 (1989) 1562–1566.
[6] W.A. de Boer, G.N.J. Tytgar, Treatment of *Helicobacter pylori* infection, Br. Med. J. 320 (2000) 31–34.
[7] A.F. Cutler, F. Havstad, C. Ma, M. Blaser, G.I. Perez-Perez, T. Schubert, Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection, Gastroenterology 109 (1995) 136–141.
[8] D.J. Evans Jr., D.G. Evans, S.S. Kirkpatrick, D.S. Graham, Characterization of the *Helicobacter pylori* urease and purification of its subunits, Microbiol. Pathol. 10 (1991) 15–26.
[9] P.R. Hawtin, A.R. Stacy, D.G. Newell, Investigation of the structure and localization of the urease of *Helicobacter pylori* using monoclonal antibody, J. Gen. Microbiol. 136 (1990) 1995–2000.
[10] A.A. van Zwet, J.C. Thijs, R. Roosendaal, E.J. Kuipers, S. Pena, J. de Graaff, Practical diagnosis of *Helicobacter pylori* infection, Eur. J. Gastroenterol. Hepatol. 8 (1996) 501–507.
[11] G.I. Perez-Perez, W.R. Brown, T.L. Cover, B.E. Dunn, P. Cao, M.J. Blaser, Correlation between serological and mucosal inflammatory responses to *Helicobacter pylori*, Clin. Diagn. Lab. Immunol. 1 (1994) 325–329.
[12] A.R. Stacy, G.D. Bell, D.G. Newell, The value of class and subclass ELISAs and antibody specificity in monitoring treatment of *Helicobacter pylori*, in: G. Gasbarrini, S. Petrolani (Eds.), Basic and Clinical Aspects of *H. pylori* Infection, Springer, Berlin, 1998, p. 159.
[13] A.M.G. Bosch, H. Van Hell, J. Brands, R.H.W.M. Schuurs, in: S.P. Pal (Ed.), Proceedings of the International Symposium on Enzyme Labeled immunoassay of Hormones and Drugs, Walter de Gruyter, Berlin, 1978, pp. 175–187.
[14] G. Gübitz, C. Shellum, Flow injection immunoassays, Anal. Chim. Acta 283 (1993) 421–428.
[15] W.R. Heineman, H.B. Halsall, Strategies for electrochemical immunoassay, Anal. Chem. 57 (1985) 129–138.
[16] D. Athey, C.J. McNeil, Amplified electrochemical immunoassay for thyrotropin using thermophilic P-NADH oxidase, J. Immunol. Methods 176 (1991) 153.
[17] S.S. Babkina, E.P. Medyantseva, H.C. Budnikob, M.P. Tyshlek, New variants of enzyme immunoassay of antibodies to DNA, Anal. Chem. 68 (1996) 3827–3831.
[18] K.R. Wehmeyer, H.R. Halsall, R. Heineman, C.P. Volle, I.W. Chen, Competitive heterogeneous enzyme immunoassay for digoxin with electrochemical detection, Anal. Chem. 58 (1986) 135–139.
[19] J. Parellada, A. Narvaes, M.A. Lopez, E. Domínguez, J.J. Fernández, J. Katakis, Amperometric immunosensors and enzyme electrodes for environmental applications, Anal. Chim. Acta 362 (1998) 47–57.
[20] T. Lim, Y. Komoda, N. Nakamura, T. Matsunaga, Automated detection of anti-double-stranded DNA antibody in systemic lupus erythematosus serum by flow immunoassay, Anal. Chem. 71 (1999) 1298–1302.
[21] C. Ruan, Y. Li, Detection of zeptomolar concentrations of alkaline phosphatase based on a tyrosinase and horseradish peroxidase bienzyme biosensor, Talanta 54 (2001) 1095–1103.
[22] EQUIPAR Diagnostici, Enzyme Immunoassay for the Quantitative Determination of IgG Class Antibodies to *Helicobacter pylori* [instruction manual], EQUIPAR Diagnostici, Rome, Italy, 2003.
[23] L. Gorton, Carbon paste electrodes modified with enzymes, tissues, and cells, Electroanalysis 7 (1995) 23–45.
[24] N. Čénas, J. Rozgaite, A. Pocius, J.J. Kulys, Electrocatalytic oxidation of NADH and ascorbic acid on electrochemically pretreated glassy carbon electrodes, J. Electroanal. Chem. 154 (1983) 121–128.
[25] G.A. Messina, A.J. Torriero, I.E. De Vito, J. Raba, Continuous-flow/stopped-flow system for determination of ascorbic acid using an enzymatic rotating bioreactor, Talanta 64 (2004) 1009–1017.
[26] F. Patolsky, Y. Weizmann, E. Katz, I. Willner, Magnetically amplified DNA assays (MADA): sensing of viral DNA and single-base mismatches by using nucleic acid modified magnetic particles, Angew. Chem. Int. Ed. 42 (2003) 2372–2376.
[27] B.A. Gregg, A. Heller, Cross-linked redox gels containing glucose oxidase for amperometric biosensor applications, Anal. Chem. 62 (1990) 258–263.
[28] M. Santandreu, F. Cespedes, S. Alegret, E. Martinez-Fabregas, Amperometric immunosensors based on rigid conducting immunocomposites, Anal. Chem. 69 (1997) 2080–2085.
[29] Z.Y. Wu, G.L. Shen, Z.Q. Li, S.P. Wang, R.Q. Yu, A direct immunoassay for *Schistosoma japonium* antibody (SjAb) in serum by piezoelectric body acoustic wave sensor, Anal. Chim. Acta 398 (1999) 57–63.
[30] G.D. Liu, J.T. Yan, G.L. Shen, R.Q. Yu, Renewable amperometric immunosensor for complement (C3) assay in human serum, Sensors Actuators B 80 (2001) 95–100.