

## PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR THE DETECTION OF *SALMONELLA ENTERICA* IN CHICKEN MEAT

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Submitted: October 13, 2004; Returned to authors for corrections: February 10, 2005; Approved: June 02, 2005

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### ABSTRACT

A panel of 13 monoclonal antibodies (MAbs) that react against outer membrane proteins of *Salmonella* Enteritidis was obtained. Two MAbs were classified as IgM, six were IgG2a, three were IgG3 and one was of the IgG2b isotype. The reactivity of the MAbs against different serovars of *Salmonella enterica* and other bacteria was investigated using an indirect ELISA. Five MAbs reacted only against *Salmonella* Enteritidis. Two MAbs presented crossed reactions with thermo-extracted antigens of *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter aerogenes*. MAb 424H presented wide spectrum of reactivity, detecting antigens of *Salmonella* belonging to serogroups B, C, D, E and G. The detection limit of different serovars of *Salmonella* in a indirect ELISA with MAb 424H varied from  $1.0 \times 10^4$  CFU/mL for *Salmonella* London to  $1.4 \times 10^6$  CFU/mL for *Salmonella* Gallinarum and *Salmonella* Typhimurium. Evaluation of the performance of the ELISA with MAb 424H in the detection of *Salmonella* in samples of chicken meat artificially contaminated revealed that the ELISA was able to detect all serovars after sample enrichment using two levels of contamination. Samples of chicken meat not artificially contaminated analysed in parallel were negative for *Salmonella* in both the conventional and the ELISA methods.

**Key words:** *Salmonella*, monoclonal antibodies, membrane proteins, foods

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### INTRODUCTION

*Salmonella* is an enterobacteria that causes one of the most dangerous foodborne infections and its presence in food represents a serious risk for public health. In Brazil, its occurrence has been observed in several types of food items, mainly of animal origin (10,21,22). In function of the risks that the *Salmonella* represents for the consumers, its detection in foods is of basic importance. The food producers, as well as the competent agencies of control, have been alert for the necessity to certify the absence of *Salmonella* in foods (3). Conventional methodology for identifying *Salmonella* is based on isolation in pure cultures, biochemical and serological tests. This methodology requires a substantial amount of time to be carried out and is expensive (8). For this reason, different systems for the rapid detection of *Salmonella* antigens in food samples

have been proposed, such as the enzyme-linked immunosorbent assay (ELISA) (20,31). ELISA-type tests use polyclonal or monoclonal antibodies or a combination of both. However, the use of polyclonal antibodies in these tests may produce a high number of false positive results due to cross reactions between antibodies present in the polyclonal serum and antigens from other bacteria or even food components (17). Many monoclonal antibodies specific for *Salmonella* epitopes have been used and provide more reliable ELISA results (7). Many of these tests are commercially available on the Brazilian market in the form of imported kits that are significantly more expensive than in their country of origin. For this reason, the development of novel reagents for diagnostics, has become economically important in Brazil, and among these, are the monoclonal antibodies, used for detection of *Salmonella* in foods. In this study we report on the production of specific monoclonal

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antibodies against *Salmonella* Enteritidis outer membrane proteins and on the evaluation of the potential of one of the MABs for use in a indirect ELISA for the detection of *Salmonella enterica* in chicken meat.

## MATERIALS AND METHODS

### Bacteria

The origin and identification of *Salmonella* strains and other Gram negative bacteria used in this study are shown in Table 1. The bacteria were cultivated in brain heart infusion broth (BHI; Difco Laboratories, Detroit, MI) for 24 hours at 37°C, streaked on nutrient agar (Difco) and subjected to Gram staining for checking purity. Pure cultures of all strains were stored at -20°C in BHI broth containing 15% glycerol.

### Antigens

#### Heat-extracted antigens

*Salmonella* and other gram-negative bacteria cultivated in BHI broth for 24 hours at 37°C, or samples of artificially contaminated chicken meat after post-enrichment in BHI for 6 hours at 37°C, were heated to 100°C for 10 minutes. The pure cultures of *Salmonella* and other bacteria had their OD<sub>600</sub> adjusted for 1.0 before being heat treated. The heat-extracted antigens were stored at -20°C until use.

#### Protein antigens from the external membrane

Proteins from the outer membrane (OMP) of *Salmonella* Enteritidis ATCC 13076 were isolated using the methodology of Filip *et al.* (9). *Salmonella* Enteritidis was cultivated in 1.5 L of BHI broth at 37°C in an orbital shaker at 150 rpm for 24 hours. The culture was centrifuged at 15,000g for 15 minutes, washed three times in 0.85% saline solution and finally resuspended in 10 mL Tris-HCl (10 mM)-EDTA (5 mM) buffer, pH 7.8 (Tris). The cells were sonicated five times with one minute pulses at 20 kcycles per second, with cooling intervals in ice for one minute, and then centrifuged at 15,000g for 30 minutes in order to remove cellular debris. The supernatant was mixed with 2.5 mL of n-laurilsarcosine (Sigma Chemical CO., St. Louis, MO) 2% (p/v) in Tris buffer and kept for 30 minutes at room temperature. After ultra-centrifugation at 300,000g for one hour the sample was resuspended in 5 mL Tris buffer and mixed with one volume of detergent solution. After one hour at room temperature the mixture was again ultra-centrifuged and the precipitate washed in 0.85% saline solution and resuspended in 5 mL Tris buffer. The material

**Table 1.** Bacterial strains used in this study.

Bacteria	Identification	Origen	Serovar
<i>Salmonella</i> Typhimurium	<sup>a</sup> ATCC 14028	<sup>c</sup> FIOCRUCZ	B
<i>Salmonella</i> Typhimurium	ATCC 13311	FIOCRUCZ	B
<i>Salmonella</i> Typhimurium	-	<sup>d</sup> LARA/RS	B
<i>Salmonella</i> Typhimurium	-	LARA/RS	B
<i>Salmonella</i> Typhimurium	-	<sup>c</sup> Cenbiot.Pel	B
<i>Salmonella</i> Agona	-	LARA/RS	B
<i>Salmonella</i> Heidelberg	-	LARA/RS	B
<i>Salmonella</i> Choleraesuis	ATCC 10708	FIOCRUCZ	C
<i>Salmonella</i> Choleraesuis	-	LARA/RS	C
<i>Salmonella</i> Hadar	-	LARA/RS	C
<i>Salmonella</i> Albany	-	LARA/RS	C
<i>Salmonella</i> Infantis	-	LARA/RS	C
<i>Salmonella</i> Infantis	-	Cenbiot.Pel	C
<i>Salmonella</i> Emek	-	LARA/RS	C
<i>Salmonella</i> Enteritidis	ATCC 13076	FIOCRUCZ	D
<i>Salmonella</i> Enteritidis	-	LARA/RS	D
<i>Salmonella</i> Dublin	-	LARA/RS	D
<i>Salmonella</i> Dublin	-	LARA/RS	D
<i>Salmonella</i> Gallinarum	-	LARA/RS	D
<i>Salmonella</i> Anatum	-	LARA/RS	E
<i>Salmonella</i> London	-	LARA/RS	E
<i>Salmonella</i> Typhi	ATCC 10749	FIOCRUCZ	G
<i>Salmonella</i> Typhi	ATCC 6539	FIOCRUCZ	G
<i>Salmonella</i> Arizona	ATCC 13314	FIOCRUCZ	G
<i>Citrobacter freundii</i>	ATCC 8090	FIOCRUCZ	-
<i>Edwardsiella tarda</i>	ATCC 15957	FIOCRUCZ	-
<i>Enterobacter aerogenes</i>	ATCC 13048	FIOCRUCZ	-
<i>Enterobacter cloacae</i>	ATCC 13047	FIOCRUCZ	-
<i>Escherichia coli</i>	<sup>b</sup> CDC 423-70	FIOCRUCZ	-
<i>Escherichia coli</i>	CDC EDL 1284	FIOCRUCZ	-
<i>Klebsiella pneumoniae</i>	ATCC 8329	FIOCRUCZ	-
<i>Klebsiella pneumoniae</i>	ATCC 10031	FIOCRUCZ	-
<i>Proteus mirabilis</i>	ATCC 15290	FIOCRUCZ	-
<i>Proteus vulgaris</i>	ATCC 13315	FIOCRUCZ	-
<i>Shigella sonnei</i>	ATCC 25931	FIOCRUCZ	-
<i>Edwardsiella tarda</i>	ATCC 15947	FIOCRUCZ	-
<i>Serratia marcescens</i>	ATCC 13380	FIOCRUCZ	-
<i>Pseudomonas aeruginosa</i>	ATCC 25619	FIOCRUCZ	-
<i>Yersinia enterocolitica</i>	-	LARA/RS	-

<sup>a</sup> American Type Culture Collection; <sup>b</sup> Center for Disease Control -USA; <sup>c</sup> Fundação Oswaldo Cruz - Brazil; <sup>d</sup> Laboratório Nacional de Referência Animal - Brazil; <sup>e</sup> Centro de Biotecnologia - UFPEL - Brazil.

was dialyzed against 0.85% saline solution and the protein concentration was determined using the Bradford method (2). The efficiency of the OMP extraction process was analyzed by SDS-PAGE electrophoresis (12).

### Monoclonal Antibodies (MAbs)

The panel of 13 MAbs was obtained according to procedures recommended by Goding (11). BALB/c mice of three to four weeks of age were immunised via intraperitoneal with the OMP from *Salmonella* Enteritidis. At the end of the immunization protocol the mouse with the highest ELISA titer of anti-OMP antibodies was sacrificed and its spleen was removed for use in the fusion process. The spleen cells were fused to Sp2/0-Ag14 cells with polyethyleneglycol (PEG; Sigma). Selection of hybridomas was carried out using Dulbecco's Modified Eagle's Medium (DMEM; Sigma) with hypoxanthine, aminopterin and thymidine (HAT). After 10 to 14 days, supernatants were screened using an indirect ELISA to detect hybridomas secreting specific antibodies for OMPs of *Salmonella* Enteritidis. The hybridomas selected were cloned twice by limiting dilution and expanded to be used in production of ascites or for storage in liquid nitrogen. Isotyping of MAbs was carried out with an ELISA test that used specific antibody class/subclass (Calbiochem, Calbiochem-Novabiochem Corporation, San Diego, California).

### MAB specificity and immunoblotting

Heat-extracted antigens of *Salmonella* and other bacteria listed in Table 1 were used in an indirect ELISA to verify MAB specificity. For immunoblotting the OMP preparations were separated in SDS-PAGE and transferred (12V/15h) to pure nitrocellulose membrane (Bio-Rad Laboratories, Richmond, CA.) (12). The membrane was blocked for 30 minutes with phosphate-buffered saline (PBS) – Tween 20 containing 5% skimm milk powder and tested with the panel of MAbs. The antigen: antibody complex was detected using an anti-mouse IgG peroxidase conjugate (DAKO Corporation, USA) and a solution of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 4-chloronaftol as substrate. After 15 minutes the reaction was interrupted with water.

### Limit of detection of the indirect ELISA

The number of cells needed to obtain a positive reaction in the indirect ELISA with MAb 424H was determined using cultures from different *Salmonella* strains grown in BHI broth for six hours at 37°C. After adjusting the OD<sub>600</sub> to 1.0 with BHI the cultures were i) diluted in base 10 in BHI, plated out in nutrient agar and incubated for 24 hours at 37°C in order to obtain a total count, or ii) diluted in base 2 in BHI broth, treated at 100°C for 10 minutes and used in an indirect ELISA as described below.

### *Salmonella* detection in artificially contaminated chicken meat

Samples of salmonella free raw chicken meat were spiked with different serovars of *Salmonella*. Each serovar was cultivated in BHI broth for six hours at 37°C, the cellular suspensions had their OD<sub>600</sub> standardized to 1.0 and were used to prepare three 25g samples, with different levels of

contamination (1-10, 10-10<sup>2</sup> and 10<sup>2</sup> - 10<sup>3</sup> CFU). Decimal dilutions from each standardized suspension were also prepared in order to carry out a viable cell count. After contamination the samples were screened for *Salmonella* using the conventional method recommended in the Bacteriological Analytical Manual – Food and Drug Administration (8) and an indirect ELISA with MAb 424H which was selected due to its greater specificity among the panel of 13 MAbs. For the indirect ELISA, 0.5 mL of each selective enrichment broth used in the conventional detection methodology were inoculated in a tube containing 10 mL of BHI broth and a post-enrichment step was carried out in a water bath shaker for six hours at 37°C. The post-enrichment cultures were heated (100°C/10 minutes) and used to coat polystyrene ELISA plates (Nunc, Brand Products, Dinamarca) with 50 µL per well for one hour at 37°C. Four wells were used for each culture, two to react with MAb 424H and two without MAb for control of non specific reactions. After coating, the plates were washed three times with PBS 0.01 M, pH 7.2, containing 0.05% Tween 20 (PBST). 50 mL of MAb diluted 1:1000 in PBST was added to each well and left for one hour at 37°C. The plates were then rewashed three times with PBST and 50 µL of protein A-peroxidase conjugate (Sigma, Saint Louis, MO) diluted 1:2000 in PBST was added and left for one hour at 37°C. After washing five times, 50 µL of chromogen/substrate (40 mg of ortophenylenediamine dihydrochloride, 40 µL of H<sub>2</sub>O<sub>2</sub> and 100 mL of phosphate-citrate buffer 0.05 M, pH 4.5) was added to each well and left to react for 15 minutes in the dark, at room temperature. The optic density (OD) was then measured at 450nm in a MR 700 microplate reader (Dynatech Laboratories, INC., Virginia). As a positive control, each plate had four wells coated with antigen that was heat-extracted from *Salmonella* Enteritidis ATCC 13076. As a negative control, four wells were coated with a mixture of antigens that were heat-extracted from negative sample cultures using the conventional method. The ELISA was considered to be acceptable when the OD<sub>450</sub> of the positive control was between 1.0 and 1.2 and the negative control was the same or less than 0.095. The ELISA cut off point, defined as the value of OD<sub>450</sub> corresponding to 96% of 20 negative samples, was 0.095.

In order to investigate the possibility of reducing ELISA analysis time, tests were also carried out with samples non-selectively enriched for six hours.

## RESULTS

### OMP antigens

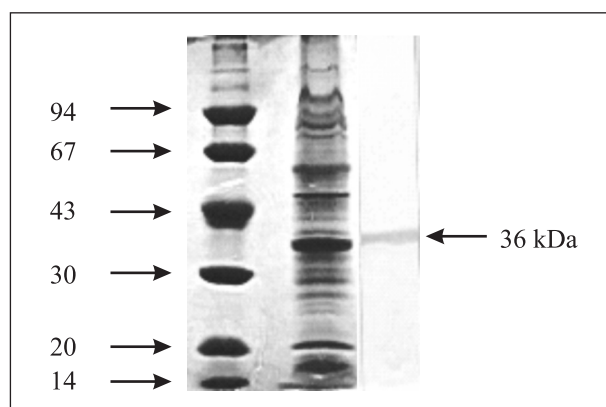
The efficiency of protein extraction from the external membrane of *Salmonella* Enteritidis was verified using SDS-PAGE. Several peptide bands could be seen after staining with coomassie blue (Sigma), among which there was a strong band with a molecular mass of approximately 36 kDa (Fig. 1). This preparation was used in the immunization protocol and in the indirect ELISA during the selection of hybridomas.

### Characterization of monoclonal antibodies

Isotyping results classified two MAbs as IgM (313D and 3210A) and the others as IgG. Among the IgGs, six were of the subclass IgG2a (MAbs 118F, 416C, 416H, 4212B, 4112C and 425D), four of the subclass IgG3 (MAbs 223C, 336D, 348C and 424H) and one of the subclass IgG2b (MAb 495B). The reactivity of MAbs with different strains of *Salmonella* and with other bacteria was investigated using an indirect ELISA as shown in Table 2. Five MAbs reacted only with *Salmonella* Enteritidis. Two MAbs showed a cross reactions with heat-extracted antigens from *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter aerogenes*. The MAb 424H showed a wider range of reactivity, detecting antigens of *Salmonella* belonging to serogroups B, C, D, E and G. A immunoblotting using a preparation of OMP from *Salmonella* Enteritidis revealed that this antibody reacts with a band of 36 kDa (Fig. 1).

### Limit of detection of the ELISA

The limit of detection for different strains of *Salmonella* in ELISA with MAb 424H was determined. In order to obtain a positive reaction it was necessary to have a minimum of  $1.0 \times 10^4$  CFU/mL for *Salmonella* London to a maximum of  $1.4 \times 10^6$  CFU/mL for *Salmonella* Gallinarum and *Salmonella* Typhimurium. For the remaining *Salmonella* serovars tested the limit of detection was  $10^5$  CFU/mL. These concentrations of bacteria in the culture media represents  $10^2$  to  $10^4$  per well in the ELISA plates.



**Figure 1.** SDS-PAGE and immunoblotting of a *Salmonella* Enteritidis OMP preparation with MAb 424H. Lane 1, molecular weight marker (Low-range – Sigma); Lane 2, fractions of OMP obtained in the extraction process; Lane 3, OMP of *Salmonella* Enteritidis blotted with MAb 424H.

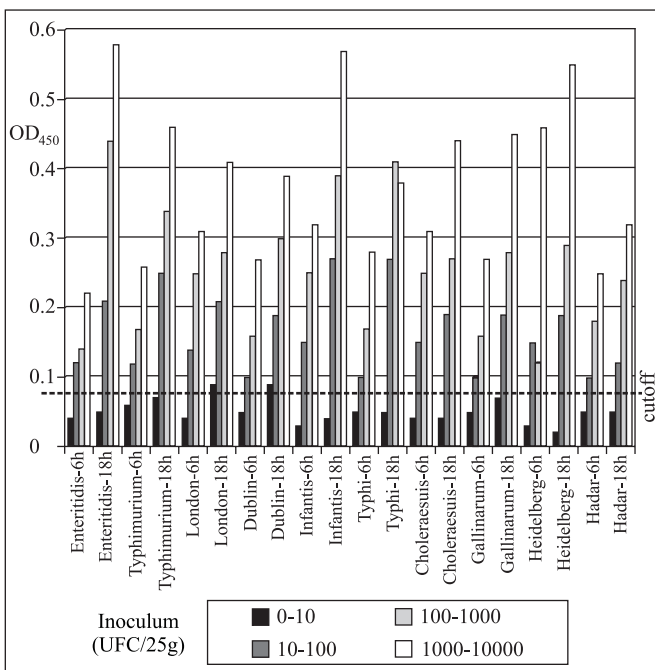
### Detection of *Salmonella* in artificially contaminated chicken meat

The ELISA using MAb 424H was able to detect the presence of all strains after two periods of sample pre-enrichment and the three levels of contamination used (Fig. 2). The analysis of

**Table 2.** Specificity of MAbs in indirect ELISA using thermo extracted antigens from salmonellae and other bacteria.

<i>Salmonella</i> (serogroup)	N° of strains tested	MAbs												
		118F	223C	313D	3210A	336D	348C	416C	416H	424H	4212B	4112C	425D	415B
Agona (B)	01	-	-	-	-	-	-	-	-	+	-	-	-	-
Heidelberg (B)	01	-	+	-	-	-	-	-	-	+	-	-	-	-
Choleraesuis (C <sub>1</sub> )	02	-	-	-	-	-	-	+	-	+	-	-	-	-
Hadar (C <sub>2</sub> )	01	-	-	-	-	-	-	-	-	+	-	-	-	-
Albany (C <sub>2</sub> )	01	-	-	-	-	-	-	-	-	-	-	-	-	-
Infantis (C <sub>1</sub> )	02	-	-	-	-	-	-	+	-	+	-	-	-	-
EmeK (C <sub>2</sub> )	01	-	-	-	-	-	-	+	-	+	-	-	-	-
Enteritidis (D <sub>1</sub> )	02	+	+	+	+	+	+	+	+	+	+	+	+	+
Dublin (D <sub>1</sub> )	02	-	-	+	-	-	-	+	+	+	-	-	-	-
Anatum (E <sub>1</sub> )	01	-	-	-	-	-	-	-	-	-	-	-	-	-
London (E <sub>1</sub> )	02	-	-	-	-	-	-	-	-	+	+	-	-	-
Gallinarum (D <sub>1</sub> )	02	-	-	+	+	-	-	+	-	+	+	-	-	-
Typhi (G <sub>1</sub> )	02	-	-	-	-	-	-	-	-	+	+	-	-	-
Arizona (G <sub>1</sub> )	01	+	+	-	-	-	-	-	-	-	-	-	-	-
Other bacteria	15	-	2 <sup>a</sup>	-	-	-	-	1 <sup>b</sup>	-	-	-	-	-	-

Positive results had  $DO_{450} \geq 0,095$ ; (+) reaction with all strains tested; (-) absence of reaction; <sup>a</sup> reaction with *Enterobacter aerogenes* e *Proteus mirabilis*; <sup>b</sup> reaction with com *Klebsiella pneumoniae*.



**Figure 2.** Results of ELISA (OD<sub>450</sub>) performed after different pre-enrichment times of chicken meat artificially contaminated with *Salmonella* Enteritidis.

aliquots from the samples of chicken that were not artificially contaminated, carried out in parallel, was negative for *Salmonella* using both ELISA and conventional methods.

## DISCUSSION

Monoclonal antibodies (MAbs) against the outer membrane proteins (OMP) of *Salmonella* were obtained. The immunogenic potential of these proteins as well as their use in the production of MAbs specific for *Salmonella* have been evaluated previously (16, 23).

Many studies have shown the production of monoclonal antibodies against to structural antigens of *Salmonella*, such as lipopolysaccharide, flagella and capsula. The use of these MAbs in ELISA tests have improved the detection of *Salmonella* in foods (16,19, 23, 28, 29). However, sometimes detection may be limited to certain strains due to the antigenic diversity of these bacteria. An example of this is the use of MAbs against flagellar antigens which are only able to identify mobile *Salmonella* (25). Another example is the cross reactivity of MAbs against somatic antigens of *Salmonella* with antigens of other bacteria due to the homology between these antigens (13, 14, 29).

For this reason, in the production of MAbs one must select specific antigens that are common to the majority of *Salmonella* serovars. The OMP used in this study resulted in a panel of

MAbs reacting specifically with *Salmonella* from several serogroups.

Five MAbs reacted only with *Salmonella* Enteritidis thereby proving their potential for use in detection tests specific for this strain which is frequently associated with foodborne infections (15). Two MAbs showed cross reactions with antigens of *Klebsiella pneumoniae*, *Citrobacter freundii* and *Enterobacter aerogenes*, which may be related to the antigenic similarity between these bacteria and *Salmonella*, as has been seen in other studies (6,16). MAb 424H was highly specific for *Salmonella*, not reacting with heat-extracted antigens from other bacteria. Moreover, as can be seen in Table 4, this MAb showed a broad range of reactivity, being able to identify strains of *Salmonella* belonging to serogroups B, C, D, E and G, which are responsible for approximately 95% of human cases of salmonellosis (27). The immunoblotting showed that MAb 424H specifically reacted with a polypeptide of approximately 36 kDa, a strongly immunogenic fraction of the OMP which represents a highly conserved epitope in different *Salmonella* serovars. This can be verified by the fact that MAb 424H has been able to react with heat-extracted antigens from all tested *Salmonella* strains.

Differences were observed on the detection limits of individual *Salmonella* serovars, using the indirect ELISA with the MAb 424H. These limits varied from a minimum of  $1.0 \times 10^4$  CFU/mL for *Salmonella* London to a maximum of  $1.4 \times 10^6$  CFU/mL for *Salmonella* Typhimurium and *Salmonella* Gallinarum, and were similar to other published ELISA (4,5,18,30). The variation observed in detection limit may be due to the variable amount of antigen present on the surface of the tested *Salmonella* serovars. This variation could also be due to differences in the antigen associated to post-polymerization modifications such as glycosylation and acetylation which makes highly specific MAbs unable to react (19).

In the experiment that evaluated the indirect ELISA with MAb 424H in the detection of *Salmonella* in samples of artificially contaminated chicken, it was shown that the assay was able to detect all strains after the two rounds of enrichment and at the three levels of contamination used. This result shows that, even in presence of the microbial competitors from the chicken meat, *Salmonella* cells reached levels that were detectable by the ELISA. Different result was found by Wyatt *et al.* (30) that showed that other microorganism in the sample interfered in the multiplication of *Salmonella* and reduced ELISA sensitivity. It should be noted that the contamination in the present study was artificial and that *Salmonella* cells did not suffer any injury which, in turn, favoured its growth even in the presence of competitive flora.

Several protocols of enrichment have been used for the isolation of *Salmonella* from foods. These protocols serve to inhibit microbial competitors while favours *Salmonella* growth (24,26). However, it has been shown that many of these protocols can affect negatively the sensitivity of the ELISA, increasing

the number of false negatives (1). In this study we were able to show that the use of an enrichment protocol with a reduced incubation time does not affect the sensitivity of the ELISA with MAb 424H. Moreover, the ELISA was able to detect *Salmonella* from a very low level of contamination which allowed us to conclude that MAb 424H is highly sensitive, being able to detect *Salmonella* while using an enrichment protocol which reduces pre-enrichment time of the sample by six hours. It should be noticed that the post-enrichment stage used was necessary for the *Salmonella* cells to reach a minimum detection level in the ELISA with MAb 424H.

In conclusion, 13 MABs specific for proteins of the external membrane of *Salmonella* Enteritidis were obtained and characterised. MAb 424H, due to its selectivity and spectrum of reactivity with various strains of *Salmonella*, was selected for use in an indirect ELISA constructed to detect salmonellae in artificially infected chicken meat and the results suggested a great potential for use in rapid immunochemical methods.

## RESUMO

### Produção e caracterização de anticorpos monoclonais para a detecção de *Salmonella enterica* em carne de frango

Foi obtido um painel de 13 anticorpos monoclonais que reagem com proteínas de membrana externa de *Salmonella* Enteritidis. Dois MABs foram classificados como IgM, 6 foram do isotipo IgG2a, três foram do isotipo IgG3 e um do isotipo IgG2b. A reatividade dos anticorpos monoclonais (MABs) com diferentes sorovares de *Salmonella* e outras bactérias foi investigada através de um ELISA indireto. Cinco MABs reagiram apenas com *Salmonella* Enteritidis. Dois MABs apresentaram reação cruzada com antígenos termoextraídos de *Klebsiella pneumoniae*, *Citrobacter freundii* e *Enterobacter aerogenes*. O MAb 424H apresentou amplo espectro de reatividade, detectando antígenos de *Salmonella* pertencentes aos sorogrupos B, C, D, E, e G. O limite de detecção de diferentes sorovares de *Salmonella* em um ELISA indireto com o MAB 424H variou de  $1,0 \times 10^4$  UFC/mL para *Salmonella* London a  $1,4 \times 10^6$  UFC/mL para *Salmonella* Gallinarum e *Salmonella* Typhimurium. A avaliação do desempenho do ELISA indireto com o MAB 424H na detecção de diferentes sorovares de *Salmonella* em amostras de carne de frango, mostrou ser possível detectar todos os sorovares após o enriquecimento das amostras, nos três níveis de contaminação utilizados. A análise de alíquotas de amostras de carne de frango não contaminadas artificialmente, feita em paralelo, foi negativa para *Salmonella* tanto no método convencional quanto no ELISA.

**Palavras-chave:** *Salmonella*, anticorpos monoclonais, proteínas de membrana, alimentos

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