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ORIGINAL ARTICLE

Evaluation of the Reveal™ Quick Test for *Salmonella* detection in raw chicken meat

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ABSTRACT. *Salmonella* belongs to the *Enterobacteriaceae* family, and is the cause of illnesses such as enteric fevers, enteritis and septicemia. Accordingly, a necessity exists of researching and developing new techniques, -which must be sensible, specific, economic and fast- for genus determination; all this related to the culture technique (official technique). In the present work, 80 samples of chicken were analyzed for Salmonella both by the Reveal™ equipment and by the culture technique with and without a pre-enrichment step (reference technique). Samples were collected in established markets in Netzahualcóyotl City and Chimalhuacán, Estado de México; and analyzed by the culture technique, from which 15 *Salmonella* strains were isolated. 14 of those strains were obtained when the pre-enrichment step was implemented. Meanwhile, using the Reveal™ equipment (new technique), the presence of 16 *Salmonella* strains was identified. The statistical method used, defined as a method of predictive values, calculates the sensitivity and specificity of the Reveal™ test in relation with the culture technique. It also determines the positive predictive value (PPV), that represents the probability that the microorganism is truly present when the Reveal™ test scores positive; and the negative predictive value (NPV), that represents the probability that the microorganim is not present when the Reveal™ test scores negative.

Key words. *Salmonella*, Reveal™.

RESUMEN. *Salmonella* pertenece a la familia *Enterobacteriaceae*, es causante de enfermedades como fiebres entéricas, enteritis y septicemias. Por esto surge la necesidad de investigar y desarrollar nuevas técnicas que sean sensibles, específicas, económicas y rápidas para la determinación del género, todo ello con respecto a la técnica del cultivo (técnica oficial). En el presente trabajo se analizaron 80 muestras de pollo, los cuales fueron analizados por el equipo Reveal™ para *Salmonella* y por la técnica de cultivo con y sin etapa de pre-enriquecimiento (técnica de referencia). Las muestras fueron recolectadas en mercados fijos de los municipios de Cd. Netzahualcóyotl y Chimalhuacán del Estado de México, las cuales fueron analizadas por la técnica de cultivo, logrando el aislamiento de 15 cepas de *Salmonella*. 14 de estas cepas se obtuvieron cuando se aplicó la etapa de pre-enriquecimiento y sólo una cuando no se aplicó dicha etapa. Mientras que por el Equipo Reveal™ (técnica nueva) sólo se logro identificar en 16 muestras la presencia del género *Salmonella*. El método estadístico de valores predictivos calcula la sensibilidad y especificidad de la prueba Reveal™ en relación con la técnica de cultivo, además de conocer el valor predictivo positivo (VPP) que es la probabilidad de que verdaderamente este presente el microorganismo cuando la prueba Reveal™ marque positivo y el valor predictivo negativo (VPN) que es la probabilidad de que esté verdaderamente ausente el microorganismo cuando la prueba marque negativo.

Palabras clave. *Salmonella*, Reveal™.

INTRODUCTION

In the last years, developed countries have been showing an increasing interest in economical losses associated to *Salmonella* infections.

Due to the importance of such genus in public health, the development of new sensible, fast, economic and specific techniques is required.[32] Since the 1960's, alternative strategies to determine the presence of microorganisms, which diminish time of analysis and increase the number of samples handled, have been developed. Among the developed techniques the one subsequently described appears: The Reveal™ assay for Salmonella that provides a fast and simple immunological test for the presumptive demonstration of the microorganism, present in clinical samples (in this research, Reveal™ was used in food) in a direct way or after an adequate pre-enrichment medium.[3] This is a test based upon the antigen-antibody recognition on a solid phase. It allows the demonstration of different *Salmonella* serovars, as it possesses antibodies against somatic antigens, specifically against its lipopolysacharide (LPS) or polysaccharide fractions, principally.[3,9,13,22,31]

This new technique is compared with the culture technique (with and without pre-enrichment), which is a reference and official technique for *Salmonella* determination, thus measuring sensitivity, specificity, positive predictive value and negative predictive values, using the method of predictive values.

MATERIAL AND METHODS

Samples. 80 raw chicken samples were collected, having been obtained from different chicken stores located within established markets of Netazahualcóyotl City and Chimalhuacán, Estado de México. Collection was carried out without any specific sampling pattern. Criteria taken into account were: 1) Choosing only fresh chicken, avoiding the purchase of samples that were subjected to refrigeration or freezing processes. 2) The vendor was asked for a particular sample (viscera, wings, legs and thighs) which was immediately put into a polyethylene bag, proceeding

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immediately to its transportation to the laboratory for analysis. 3) General conditions in which samples were found at the moment of its collection such as item temperature and exposure to air, sun and dust were recorded.

Preliminary assays. Before Reveal™ for *Salmonella* was used, three preliminary tests were realized. The first test consisted of choosing a control strain, for which 2 strains were used: *Salmonella typhi* ATCC 7251 and *Salmonella enteriditis* Group B (wild strain) which were cultivated in assay tubes containing BHI medium for 6 h at 37ºC. Afterwards, the Reveal™ test was applied, recording the results.

The second test was the election of both the enrichment medium and incubation time necessary in order to apply the Reveal™ test, for which each control strain was inoculated in a tube containing tetrathionate medium with iodide-iodided, and in another tube with selenite cystine medium. These were incubated at 45ºC for 6, 8 and 24 h, before the Reveal™ was applied. Once the tubes reached 24 h of incubation, the culture technique was implemented, to see if it could be possible to obtain the cultivated strain again.

The third preliminary test consisted in determining the minimal bacterial concentration detectable by the Reveal™ test for *Salmonella*. An 18-hour culture of the *Salmonella typhi* ATCC 7251 strain was used. The culture was adjusted to tube number one of the MacFarland nephelometer, then making decimal dilutions with sterile saline solution (from 10^8 to 10^{10} bacteria/ml) and applying the Reveal™ test.

***Salmonella* determination in raw chicken meat by the conventional culture technique.** Collected samples were analyzed using the conventional culture technique.

Pre-enrichment. The chicken was chopped in aseptic conditions taking 25 g into a 500 ml flask containing 225 ml of sterile lactose-enriched medium. The samples were incubated from 18 to 24 h at 37± 2ºC.

Enrichment. Whenever the sample did not require any pre-enrichment, 15 g of chopped viscera or chicken meat were placed in a flask containing 125 ml of tetrathionate medium with 2 ml of iodide-iodided in aseptic conditions. Another 15 g were placed in a flask containing 125 ml of selenite-cystine medium. Both cultures were incubated for 24 h at 43ºC. Whenever the sample had been subjected to pre-enrichment, 1 ml of the pre-enrichment culture was transferred to a screw-cap tube containing 10 ml of tetrathionate medium with 0.2 ml of iodine-iodized, and another ml transferred to a screw-cap tube with 10 ml of selenite-cystine medium. Both tubes were incubated for 24 h at 43ºC.

Isolation. From each flask or tube with enrichment medium, the three following plates were inoculated on Bismute-sulfite Agar, Hektoen Enteric Agar and Mac-Conkey Agar. The plates were incubated at 43º C for 24 h. Whenever the plates did not show typical colonies or growth was not observed, the plates were incubated for an additional 24 h.

Biochemical identification. 1 or 2 typical colonies were selected from each plate. The colony suspected of being of the *Salmonella* genus was carefully inoculated in a tube with TSI agar and in another tube with LIA agar by both pinching and striate inoculation. These tubes were incubated at 35±2ºC for 24 h.

Serological identification. After identifying the TSI and LIA tubes, which presented a characteristic reaction to *Salmonella*, 2 drops of sterile saline solution were placed in a slide with a small portion of the TSI developed culture, adding afterwards a drop of polyvalent antiserum and mixing. A clear agglutination in the slide was considered a positive result, while a null agglutination was considered a negative one.

The *Salmonella* strains obtained by the culture technique were sent to the laboratory of the Instituto Nacional de Referencia Epidemiológica, S. S. A. to be typified.

Implemented technique for the Reveal™ test for *Salmonella.*

Pre-enrichment. The chicken was chopped in aseptic conditions taking 25 g into a 500 ml flask containing 225 ml of sterile lactose enriched medium. The samples were incubated from 18 to 24 h at 37± 2ºC.

Enrichment. Whenever the sample did not require any pre-enrichment, 15 g of chopped viscera or chicken meat were placed in a flask containing 125 ml of tetrathionate medium with 2 ml of iodine-iodized in aseptic conditions. Another 15 g were placed in a flask containing 125 ml of selenite-cystine medium. Both cultures were incubated for 6, 8 and 24 h at 43ºC.

Whenever the sample had been subjected to pre-enrichment, 1 ml of the pre-enrichment culture was transferred to a screw-cap tube containing 10 ml of tetrathionate medium with 0.2 ml of iodine-iodized, and another 1 ml transferred to a screw-cap tube with 10 ml of selenite-cystine medium. Both tubes were incubated for 8 h at 43ºC.

Application of the Reveal™ test for *Salmonella*. Each bag of the Reveal™ test for *Salmonella* was incubated for 5 min at 37ºC. Then the test was taken from its bag to be added with 3 drops of the sample enriched medium (previously incubated 6, 8 and 24 h). They were put back into their bags, which were closed and incubated for 20 minutes at 37ºC horizontally.

Both negative and positive Reveal™ equipment blanks were incubated likewise. Once incubation time was over, the individual tests were taken from their bags and the results recorded.

RESULTS AND DISCUSSION

In Table 1 the results for the first preliminary test are represented. This test was designed in order to determine the

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control strain to be used in both the culture technique and the Reveal™ test. Two strains were tested, one out of a collection, the other, a wild one. The strains were, accordingly, *Salmonella typhi* ATCC 7251 and *Salmonella enteriditis* Group B. We found that the *Salmonella typhi* strain yielded the best results when the test was applied. Nevertheless, it is pertinent to point out that when the strain was grown in a tetrathionate enriched medium, the purple line in the test zone was lighter and defined -this due to the tone contrast with the running surface, which remained white due to the white color of the tetrathionate medium. In contrast, when the selenite-cystine medium was used, a change in the color of the running surface from white to salmon (the medium's color), having as a consequence a loss in the definition of the purple band.

On the second preliminary test, as indicated in Table 2, the time for the best Reveal™ test result for microorganism identification (*Salmonella typhi*) was tested. This was 8 h, since by the 6th hour there was no presence of the genus. With *Salmonella enteriditis* Group B, the Reveal™ test was not able to show the presence of the microorganism when it was enriched in the selenite-cystine medium for 6, 8 or 24 h. Although the presence of this microorganism was already pointed out when it was enriched with the tetrathionate medium for 24 h, given that no presence of the strain was observed either at 6 or 8 h. It is worth to mention once again that the when the tetrathionate and the selenite-cystin tubes had finished their 24 h incubation time, the culture technique was continued until it was over, as a way to re-isolate both strains initially inoculated in each tube. With this, the Reveal™ test's difficulty to detect *Salmonella enteriditis* Group B, especially when the strain comes from a selenite-cystine enriched medium, is confirmed, because the presence of the microorganism in the medium was observed when the strain was recuperated with the culture technique.

The third test was implemented in order to determine the minimal amount of bacteria detectable by the Reveal™ test. To accomplish this, only the *Salmonella typhi* ATCC 7251 collection strain was employed. Results are shown in Table 3. The minimal amount of bacteria detected was 10^5 bacteria/ml.

Regarding a test called Single Step Test Device, it is reported that it is feasible to detect the presence of minimal concentrations of 10^5 UFC/ml, using *Salmonella typhi*.[17] It is noteworthy that both tests are equal in the sense that both of them were designed for the rapid detection of Salmonella in feces; this is besides the fact that they are produced by the same corporation (AMPCOR Diagnostic, Inc.).[9,30]

Table 1. Results obtained with the Reveal™ Test for control strain election.

*Reveal TM**	*Controls*	*Positive*	*Negative*	*Observations*
Salmonella typhi ATCC 7251	Positive	Positive	Negative	The line obtained was clearer and more intense, like the positive control.
Salmonella enteritidis Group B	Positive	Positive	Negative	The line obtained was less clear and less intense with respect to the positive control.

*after 6 h of incubation

Table 2. Results obtained in the election of the enrichment medium and incubation time for the Reveal™ test.

Type Strains	*Enrichment Medium*	*Incubation Time (h)* 6	8	24	*Observations*
Salmonella typhi ATCC 7251	Tetrathionate	Negative	Positive	Positive	The line obtained after 8 h was similar to the 24 h one (clear and intense).
	Selenite-Cystin	Negative	Positive	Positive	The line obtained after 8 h was simi lar to the 24 h one, but it was neither as intense nor as clear as the one obtained with the tetrathionate medium.
Salmonella enteritidis Group B	Tetrathionate	Negative	Negative	Positive	The line obtained was neither clear nor intense.
	Selenite-Cystin	Negative	Negative	Negative	No lines were obtained but the ones that validate the test.

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Table 3. Results of the minimal bacterial number detected by the Reveal™ test for *Salmonella*.

Estimated bacterial number for Salmonella typhi *ATCC 7251 (bacteria/ml)*	*Result of the Reveal™ Test*
10^8	Positive
10^7	Positive
10^6	Positive
10^5	Positive
10^4	Negative
10^3	Negative

Table 4. Viscera and chicken meat samples.

Viscera samples (n = 25)	*Chicken meat samples (55)*
Livers (19)	Legs (25)
Gizzards (5)	Thighs (21)
Gut (1)	Wings (6)
	Coccyx (3)

Using the information provided by the producer, in the case of the Single Step Test Device, it mentions that it has the capability of detecting the presence of concentrations of 10^3 UFC/ml. for *Salmonella typhi* and of 10^4 UFC/ml. of *Salmonella typhimurium.*[3]

From the 80 samples collected, the kind of analyzed samples are indicated in Table 4. The conditions in which the chicken was found at the time of its sale were deficient (temperatures from 18 to 21ºC, exposed to sun, dust and flies, apart from being handled inadequately),[16] allowing all possible risks of bacterial proliferation and illnesses related to chicken meat to reappear.[37]

Chicken samples were collected in 16 samplings as shown in Table 5. In the first sampling (consisting of 2 liver samples, 1 of guts and 1 of coccyx), using enrichment times of 6, 8 and 24 h, a Reveal™ test was implemented by the end of each incubation time in both tetrathionate and selenite-cystine media. At this point, it was observed that, when applying the test after 6 and 8 h incubation times, for both enrichment media, only one purple band in the control zone was obtained indicating the absence of *Salmonella* in the sampling as a negative result. This was not the case after 24 h of incubation for both pre-enrichment media, because there was a dispersion of the colored complex of the test, resulting in the absence of the purple band in the control zone, pointing the result as doubtful, even when the test was repeated.

The possible explanation for the test behaving like this, is the probably high numbers of associated biota existing in the sample, as it is known that although the media are for *Salmonella* enrichment, the growth of other bacterial genera is possible. In the case of the tetrathionate medium, *Proteus* has proven to be capable of growing in it, for it can reduce both the tetrathionate and thiosulfate present in the medium. Other genera capable of growing in such a medium are *Escherichia coli* and *Shigella.*[27] Regarding the selenite-cystine medium, *Arthrobacter* is capable of growing in it.[3]

This was confirmed later, when observing the massive growth in plates of Mac-Conkey and Hektoen's enteric agar, as well as a moderate growth in bismuth-sulfite agar plates. In these media, besides expecting *Salmonella* to grow, it is possible to witness the growth of positive and negative lactose bacteria in Mac-Conkey agar; *Proteus*, *Pseudomonas* and *Citrobacter* can grow in Hektoen's enteric agar; and *Enterobacter*, *Proteus*, *Citrobacter*, *Klebsiella* and *E. coli* can grow in bismuth-sulfite agar.[7,24]

The samples collected during the second sampling were analyzed again, using both enrichment media. The only changes were in incubation times, and these were 6, 7, 8, 9 and 10 h. This was made in order to determine the time at which the running of the complex in the Reveal™ test begins, for it may invalidate results or present them as doubtful. By hours 6 and 7, a negative outcome was obtained for both enrichment media with the Reveal™ test; while by hours 9 and 10, the outcome was a running of the colored complex, similar to that obtained with the first sampling after 24 h of incubation. Based on these results, we decided to

Table 5. Number of samples collected in each sampling.

Sampling number	*Number of samples*
1	4
2	7
3	5
4	5
5	3
6	5
7	5
8	5
9	5
10	5
11	5
12	5
13	5
14	5
15	6
16	5
Total	80

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apply the Reveal™ test for *Salmonella* after an 8 hour enrichment period in tetrathionate medium, beginning with the third sampling.

When we finished analyzing the fifth sampling, it became evident that only one strain of *Salmonella* had been identified and isolated, and this was done with the culture technique (sample number 20, liver). Therefore we decided to apply a pre-enrichment step with lactose enriched medium to the subsequent samples for 24 h (as stated in the Norma Oficial Mexicana).[26] It is noteworthy that although it is not necessary to implement a pre-enrichment step for raw chicken meat, the decision to use one answered to the fact that a larger number of identifications with the Reveal™ test as well as more isolations of *Salmonella* genre with the culture technique were found.

This pre-enrichment is used to increase the *Salmonella* recuperation percentage, by allowing the sub-lethally damaged cells to repair. This damage could be consequence of any exposure to unfavorable conditions given along the handling of the chicken, such as refrigeration, which could increase the susceptibility of the cells. Therefore, not including an enrichment step could result in the non-detection of those cells which can recover themselves and that might cause an infection if the chicken is not handled properly.[1,15,23,36] Although it is also reported that *Salmonella* is inhibited via competitive growth in the pre-enrichment medium by other genera such as *Pseudomonas* and Gram positive bacteria.

Some authors recommend using a brief period of 6 to 8 h for *Salmonella* to recover, thus not favoring the growth of the associated biota.[5,8]

Table 6. Decision matrix to analyze data.

		Reference Technique	
		Positive	*Negative*
New Test	Positive	A True positives	B False positives
	Negative	C False negatives	D True negatives

Table 7. Results obtained with both, the culture technique without pre-enrichment and the Reveal™ test in a 2 X 2 contingency table.

			Culture technique (without pre-enrichment)		
			Positive	*Negative*	*Total*
Reveal™ test for	*Salmonella*	Positive	0	0	0
		Negative	1	23	24
		Total	1	23	24

Therefore, by the sixth sampling, (numbers 25 to 80) samples were subjected to a pre-enrichment step. In this manner, 15 *Salmonella* isolations were obtained by the culture technique, and 16 samples were positive for the Reveal™ test.

Once these data were obtained, we applied the method of predictive values. This method allows us to answer the first two immediate questions that emerge when using a new test:

- If the microorganism is present, what is the probability of a positive result in the test? and,
- If the microorganism is not present, what is the probability of a negative result in the test?

The answer to the first question is determined by the sensitivity of the test, and the second one by its specificity. Then it is necessary to consider:

a) If the new test gives a positive outcome, what is the probability of the microorganism really being present?
b) If the test gives a negative outcome, what is the probability of the microorganism not to be present at all?

The answer to both questions rests upon the positive predictive value (PPV) and upon the negative predictive value (NPV), respectively.

The obtained data were registered in a two-income table or decision matrix for its numeric representation, as shown in Table 6.

The way to calculate the parameters is the following: The sensitivity is equal to the number of true positives divided by the number of those who truly had the microorganism, times 100 (A/A+C X 100).

The specificity is equal to the number of true negatives divided by the number of those who truly did not have the microorganism times 100 (D/D+B X 100) .

The positive predictive value (PPV) is equal to the number of true positives divided by the total number of positives, times 100 (A/A+B X 100).

The negative predictive value (NPV) is equal to the number of true negatives divided by the total number of negatives, times 100 (C/C+D X 100).

Using the results obtained from the first five samplings, where a total of 24 samples were analyzed using both the culture technique (without pre-enrichment) and the Reveal™ test. The distribution according to the method of predictive values is shown in Table 7.

Also, in Table 8 the analysis results of the 56 samples are shown. These samples were collected along from 11 samplings, and were analyzed both by the culture technique (with pre-enrichment) and by the Reveal™ test.

In general, when analyzing the obtained results, only 15 *Salmonella* isolations were obtained with the culture tech-

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nique out of a total of 80 samples. Of those samples, 14 were obtained when a pre-enrichment with lactose enriched medium was implemented.

The low number of isolations might be due to the time of the year during which the study was carried out, since it is reported that such genre may be isolated and detected in any time of the year, but the increase in the number of cases begins in May, reaching its peak by July and August, with a decrease starting on September.[12,18]

On the other, we must consider, not only the time of the year during which the study was done, but the general handling of the animals from birth to death, as much as their transportation and selling, because this way a large number of extraneous microorganisms are incorporated to the sample, considerably increasing the microbial biota which compete with *Salmonella*.

The largest number of isolations occurred when the chicken samples were sold at 21ºC agreeing with the reports in which *Salmonella* is mentioned to keep a slow growth between 18 and 25ºC.[14,21,28]

We must add that the *Salmonella* genre is a poor competitor, for it is strongly inhibited by associated biota such as *E. coli* and lactic bacteria, which produce lactic and acetic acids and diacetyl,[15,18,21] thus favoring the minimal growth of *Salmonella*, because the number of bacteria lowers as the medium's pH does.

When the pH is between 6.8 and 6.9, the microorganism multiplies actively: it is known that pH values below 4.1 and above 9.0 limit its growth.[18,21]

Table 8. Results obtained with both, the culture technique with pre-enrichment and the Reveal™ test in a 2 X 2 contingency table.

		Culture technique (with pre-enrichment)		
		Positive	*Negative*	*Total*
Reveal™ test for *Salmonella*	Positive	7	9	16
	Negative	7	33	40
	Total	14	42	56

Table 9. Parameters calculated for the Reveal™ test with respect to the culture technique with and without pre-enrichment.

Parameters	*Culture technique without pre-enrichment*	*Culture technique with pre-enrichment*
Sensitivity	0 %	50 %
Specificity	100 %	78.57 %
Positive Predictive value	0 %	43.75 %
Negative predictive value	95.83 %	82.50 %
Prevalence	4.34 %	25 %

The parameters calculated to evaluate the Reveal™ test, when compared to the culture technique with and without pre-enrichment, are shown in Table 9.

In a diagnostic test, as in the case of the Reveal™ equipment for *Salmonella*, the closer to 100% in sensitivity and specificity the test gets, the greater its ability to discriminate a sample that contains the microorganism from one which does not. A low probability (less than 50%) indicates a difficult of the equipment to detect accurately such genre.

When analyzing the obtained results from the samples without pre-enrichment, 0% sensitivity was obtained, because the Reveal™ equipment gave a negative result in the only sample for which the culture technique had a positive outcome. Thus, the new test was not able to detect a positive sample from the true positives. In the stage of the study with pre-enrichment, the sensitivity was of 50%, even when the number of *Salmonella* isolations increased. A high probability of finding false negatives remains, that is, when the microorganism is isolated by the culture technique, the Reveal™ equipment does not detect it, which could be quite dangerous.

Regarding the specificity of the equipment, scores of 100% and 78.57% were obtained when compared with the culture technique without and with pre-enrichment, respectively. The first percentage is the ideal for a test, but it is not possible to evaluate the Reveal™ equipment based on this. On the other hand, the second score is low, thus the probability of crossed reactions with other bacteria is considerable.

As a whole, both parameters allow us to evaluate the performance of the test in posterior assays from an analytical perspective, because they allow the prediction of the outcome of a particular test, taking as a reference another analytical method (that is, the standard or reference technique).

The predictive values are important from a post-analytical view, for they let us predict if the result obtained in a particular assay has any validity when compared to the result obtained from the standard method. So, if the values are inferior to 50%, it is very improbable that the outcome of the assay is valid, and on the contrary, the closer the values are to 100%, the more reliable the assay is.[9,10]

The positive predictive value of 0%, obtained when comparing the Reveal™ test against the culture technique without pre-enrichment, indicates that when the outcome of the test is positive, the probability of the microorganism to truly be present, is 0%, a null probability. When comparing the Reveal™ test against the culture technique with pre-enrichment, the positive predictive value turned out to be 43.75%.

Nevertheless, it is not enough to consider it valid value for such a parameter. With the negative predictive value, a probability of 95.83% was obtained when comparing the Reveal™ test against the culture technique without pre-enrichment, and of 82.50% when compared against the culture technique with pre-enrichment. Both probabilities are

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large, pointing out that when the Reveal™ test gives a negative outcome, the probabilities for the microorganism to be truly absent are 95.83 and 82.50%.

The culture method compared to immunological tests has a difficulty rarely taken into account. That is, the cultures only allow the detection of viable microorganisms, while the immunoassays can detect the presence of viable organisms, non-viable ones, and/or of fractions which are antigenic to them. This possibly explains why the test detected the presence of *Salmonella*, while the culture technique did not.

Therefore, the results obtained, as much as the determined parameters, must be taken with caution, due to the difference above noted.

As a complementary part to this work, once the analysis of the samples was finished, the identification of the *Salmonella* strains isolated by culture was done by the INDRE (Instituto Nacional de Referencia Epidemiológica, SS, México), in order to know which was the species involved.

The species found in the 15 isolated samples was *Salmonella enteriditis*, somewhat confirming several reports emitted worldwide, where the considerable increase in such species is noted.[2,4,17,19,25,35,36]

Also, it is confirmed that the most commonly isolated strain in fowl and its products (including eggs) corresponds to *Salmonella enteriditis*, of which the animal gets contaminated at the time of birth.[4,19,20,2934,35] The isolation of this microorganism in chicken is done generally at the ovaries and oviducts.[34] Salmonellosis, particularly paratyphoidea has a huge economical importance in national aviculture: Only in the USA, *Salmonella enteriditis* represented the 6% of the isolated serotypes in sprouts by alimentary contamination in the 80s. In 1990 it constituted 21% of isolated serotypes.[11]

The minimal bacterial concentration detectable by the Reveal™ test was of 10^5 bacteria/ml.

The Reveal™ test for *Salmonella* presented limitations in detecting the presence of the *Salmonella* genre in raw chicken meat, therefore a low sensitivity and specificity were obtained.

Better results were obtained when the tetrathionate medium was implemented in the enrichment stage, therefore it is recommended for further studies.

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