EXPERIMENTAL STUDY ON THE THERMAL BEHAVIOUR OF A CONTAINER FOR IMMUNOBIOLOGICAL

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Abstract. This work presents the development of a Container which cares of a demand of the PNI- the National Immunization Program, through the Chain of Cold, which standardizes all the procedures for conservation, manipulation, distribution and transportation of vaccines in low temperatures, since their producing in laboratory up to the moment of using in places that, sometimes, don't have electrical energy. This Container has easy manuscript and aims to satisfy the necessity of simple and practical form with low operational costs. This Container was specifically developed for the Vaccine of Influenza Virus Inactivated against the Grippe. The choose for this vaccine relies on the facts of being close to the consuming market and having temperature of conservation oscillating between 2ºC and 8ºC without loss of efficiency for a desirable time of 8 hours. This band of temperature is also applied to most of the excessively immunobiological vaccines, so it would make easier the adaptation of this project for other vaccines. Initially we monitored the thermal behaviour of the boxes of transportation and conservation of imunobiológicos currently used in the vaccination practice, through simulation in real situations as the Real Test. Later we search the thermo physical properties of all the material used in the project and made the sizing of container in question. Some simulations in real conditions of vaccine using were made and the thermal boxes performance was measured. After projected and constructed the Container's archetype, it was exhaustingly tested in different ambient conditions as the Tests 1, 2 and 3. The results of the experimental tests are presented and discussed in this work and the archetype has corresponded to the expectations regarding the time and foreseen temperatures of conservation of the selected immunobiological.

Keywords: *Container, immunobiological, temperature.*

1. INTRODUCTION

From the early days of humanity morbimortality rates (mortality caused by disease) has been very high, thus it has emerged with the evolution of the biological sciences a pressing need for scientific research geared to the development of methods and parameters to reduce this rate.

This research effort led to the creation of vaccines and immunobiologicals, defined as microorganisms used to provoke active immunity states against certain infectious diseases. The vaccines are made of attenuated viruses, producing antibodies and antitoxins. In order to ensure that immunobiologicals preserve their initial characteristics, it is vital that they are refrigerated at temperatures of between 2 and 8 º C, which can preserve their immunobiological capacity. The immunobiologicals are thermolabil and as such they will deteriorate after a certain time of exposure to temperatures inadequate to their preservation, since heat will speed the process of inactivation of the immunogenic components. The Cold Chain Manual, written by "Funasa (2001)", includes the norms and procedures for all the processes involved in the storage, conservation, handling, distribution and transportation of immunobiologicals of PNI (The National Immunization Program). The procedures described in this manual dictate that the immunobiologicals must be placed and transported in polystyrene or polythene boxes, previously encircled by recyclable ice spools, and that during vaccination campaigns ice cubes of scraped ice must be used inside plastic bags. This is a completely unacceptable practice, once the plastic bags with ice, due to their irregular shape, make it difficult for the immunobiologicals to be duly ensconced, and the empty spots inside these bags compromise thermal insulation, thus jeopardizing temperature maintenance. On top of all that, when the professional opens the box to handle the vaccine,

he or she will be exposing all the other vaccines to the heat that comes from outside, and which will speed the heating process.

Studies on the conservation of immunobiologicals have been carried out. “Sbeghen (1998)”, concluded that the system used for the conservation of the vaccines is vital if the quality of the vaccines is to be guaranteed; and the main cause for the loss of vaccines in the public vaccination programs in the US is the inadequacy of Cold Chain.

“Rizzo *et al*. (1990)” observed in an experiment that photo sensibility and thermo stability are determining factors in the fall of potency when the vaccines are exposed to the incidence of light. Exposure of the vaccines to heat leads to low stability and reduction of homogeneity.

“Malinverni (2003)”, concluded that any breach in this Cold Chain may cause the vaccine to lose its immunization property, since there is a direct correlation between the effectiveness of the vaccines and the maintenance of the cold chain at every stage, that is, form their production to their storage, to transport and handling.

The mode of conservation of biological products, according to “Hoffmeister (2001)”, must be as adequate as possible, because the temperatures outside the conservation band will affect the effectiveness of the vaccine and cause localized reactions in the patients.

After experimental tests, “Albas *et al*. (2001)” published that the anti-rabies vaccine produced in the brains of newly-born mice practically lost its capacity to induce neutralizing anti-bodies when frozen. Such conditions have rendered the vaccine useless for pigs and most probably for human beings.

The purpose of the project was to research into the reliability of the current procedures, seeking the likely optimization of the process and securing the maintenance of the quality of the vaccines as well as the enhancement of the process as a whole by implementing vaccination campaigns.

The container was developed, built and tested in order to assist the Cold Chain professionals, by guaranteeing the quality and the effectiveness of the immunobiologicals, rendering handling easier, promoting functionality, operationally and low implantation costs.

2. METODOLOGY

Today, the Cold Chain is used in the transport and conservation of conventional vaccine boxes made of polystyrene or polyurethane, surrounded by recyclable ice spools and filled with the packaging containing the vaccines.

Taking as a basis the Cold Chain Manual, a polystyrene container was designed for the maintenance of the temperature inside it, at the band needed for the effective conservation of the vaccines, and keep the thermo condition of each vaccine unaltered while they are being transported and while the vaccines are being inoculated.

So that the inner space is better used, holding the largest possible number of units, the individual thermal insulation of each corner of the container was carefully studied as well as the improvement of the inner layout of the box.

The inner space of the container has two polystyrene grids for the vertical storage of the vaccines, making it possible for individual caps to be used, so that one vaccine can be handled without altering the temperature of the others. It also has four thermal ice walls that work as barriers, preventing heat from the outside from entering the interior of the box.

2.1. Experimental procedure

2.1.1. Real Test

Real Test procedure simulates the methods used nowadays on extramural vaccination procedures.

Through this is expected a direct analysis on the thermal behaviour of the polystyrene boxes used, once the temperature has a direct influence on the immunobiological efficiency. The proposed environment to this simulation is closed, non-refrigerated and without incidence of sun light.

Four experiments were made, aiming to check the efficiency of the vaccination procedures used nowadays on vaccination campaigns. Seven thermocouples were used in syringes with water. It was defined one thermocouple for the environment temperature monitoring during the experience. Ice cubes were prepared for the packing in plastic bags, at the same time the temperature stabilization of 40 vaccines between 2ºC and 8ºC was monitored through a data acquisition board. Among this units were the ones containing the thermocouples. The polystyrene box’ assembly respected the usual procedures, in other words, the vaccines and plastic bags with ice cubes were placed casually in the polystyrene box. However, the vaccines with thermocouples were kept on the same position so we could be sure on the thermal behaviour on the spot they were.

The spots chosen on the system were the box extremities and centre, as it can be observed on Fig.1. We believe that through the thermal behaviour analysis on this spots it is possible to analyse the thermal behaviour in the all box by symmetry.

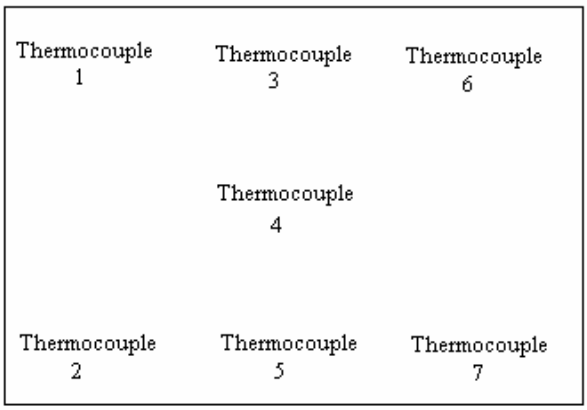

Figure 1. Position of the immunobiological with thermocouples for the Real Test

2.1.2. The container projected

2.1.2.1. Description of the container

The developed Container has rectangular shape, measuring: 610 mm long, 430 mm wide and 210 mm high and capacity for 32 units. The prototype is made of P2 polystyrene plate, with a density of 220 kg/m^3 and 30 mm thick, according to Fig. 2.

2.1.2.2. Description of the thermal walls

The dimensions of the thermal walls are similar to those of the recyclable ice spools used today in the vaccination practices, being 510 mm long, 120 mm high e 20 mm thick, as indicated in Fig. 2.

2.1.2.3. The experimental tests on the projected container

The tests were conducted in three different environments, in conditions that are as usual as possible in terms of real current practices, as follows:
Test 1: non-refrigerated closed environment without incidence of sunlight.
Test 2: open air in the shade.
Test 3: open air with direct incidence of sunlight.

2.1.2.4. Position of the thermocouples in the projected container

Since the symmetry of the developed container is xy; its thermal behaviour was analyzed by means of the study of one fourth of the system. This is the reason why the thermocouples were positioned as shown in Fig. 2.

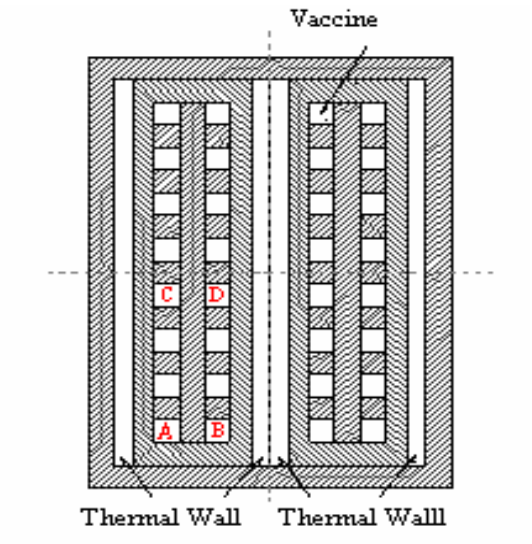

Figure 2. A, B, C and D position of the thermocouples during the Tests 1, 2 and 3

2.3.3. Data collection

The data on the temperature of the system were collected by means of a Tpaq 100 data collection plate, produced by Datapaq Inc, capable of storing 16.000 measurement values, through 8 data collection channels. This plate operates with thermocouples of the K type, have a resolution of 0.1º C and precision of ± 1º C.

2.3.4. Methodology for the calibration of the thermocouples and error

Once a contact digital thermocouple had been defined as standard, a mixture of ice and water was prepared, and 15 (fifteen) temperature measurement were taken with 04 (four) thermocouples used in the experiment and connected to the DATAPAQ data collection plate.

Table 1 shows the error in the measurements of each thermocouple, by taking as a basis the behaviour of the thermocouple defined as being standard. The variations are not very significant, once the temperature for the conservation of the vaccine may fluctuate between 2 and 8º C.

Table 1. Error in the measurement of the thermocouples by taking as a basis the standard thermocouples.

THERMOCOUPLES									
TIME (min)	STANDARD	TEMPERATURES °C							
		POSITION A		POSITON B		POSITION C		POSITION D	
		I	IM	I	IM	I	IM	I	IM
5	0.90	0.30	-0.60	0.60	-0.30	0.50	-0.40	0.40	-0.50
10	1.70	1.20	-0.50	1.40	-0.30	1.20	-0.50	1.40	-0.30
15	2.20	2.00	-0.20	2.20	0.00	2.10	-0.10	2.10	-0.10
20	2.90	2.90	0.00	3.00	0.10	2.90	0.00	2.90	0.00
25	3.60	3.70	0.10	3.90	0.30	3.80	0.20	3.90	0.30
30	4.50	4.90	0.40	5.00	0.50	5.00	0.50	5.00	0.50
35	5.30	5.70	0.40	5.80	0.50	5.70	0.40	5.80	0.50
40	6.40	6.60	0.20	6.80	0.40	6.60	0.20	6.70	0.30
45	7.20	7.50	0.30	7.60	0.40	7.60	0.40	7.60	0.40
50	8.10	8.00	-0.10	8.00	-0.10	7.90	-0.20	8.00	-0.10
55	9.00	8.80	-0.20	8.90	-0.10	8.90	-0.10	8.90	-0.10
60	9.80	9.40	-0.40	9.40	-0.40	9.40	-0.40	9.40	-0.40
65	10.40	10.20	-0.20	10.30	-0.10	10.20	-0.20	10.30	-0.10
70	11.50	10.80	-0.70	10.90	-0.60	10.80	-0.70	10.90	-0.60
		E_{max} = 0.70		E_{max} = 0.60		E_{max} = 0.70		E_{max} = 0.60	
		E_{min} = 0.00		E_{min} = 0.00		E_{min} = 0.00		E_{min} = 0.00	
		E_{mean} = 0.30		E_{mean} = 0.30		E_{mean} = 0.30		E_{mean} = 0.30	

IT BEING: I = Uncertainty IM = Mean uncertainty

3. RESULTS

In the presentation of the results the temperature and time variables were non-dimentionalised, having behaved in this way, as a whole, during the monitoring process in the Real Test, Tests 1, 2 and 3 proposed:

$\theta = (T - T_\infty) / (T_V - T_\infty)$ represents temperature; T_∞ room temperature; T the temperature of the vaccine at a certain moment and T_V the temperature of the vaccine at the beginning of the monitoring process.

$\delta = t / t^*$ represents time; t represents the period of time of conservation of the vaccine at the interval of 2 to 8 º C, and t * the desirable time of 8 hours. This non-dimensional represents the parameters that indicate the performance of the system.

The diagrams show the mean temperatures obtained from the vaccines during the monitoring process. This parameter satisfactorily represents the thermal behaviour of the vaccine, the standard deviation being negligible and the means, modes e medians having practically the same values.

3.1. Real test results

Through the data presented on Tab. 2, we can verify that the average room temperature was 23ºC and the initial immunobiological average temperature at the beginning of the monitoring was 6ºC, so the top temperature to guarantee the immunobiological efficiency (8ºC) is reached when the values θ_{min} approximate to 0.88 (adimensional in which the temperature of the immunobiological reaches its required maximum temperature for maintenance of the efficiency); as we observed on Fig. 3, these values were reached in a very short period of time, and up to the temperature of 8ºC the immunobiologicals get in a process of loss of efficiency.

The curves on Fig. 3 show that the time of stability of the immunobiologicals on the requested temperature was too short. This is pointed, specially, by the general average curve designed in the limits between θ_{max} and θ_{min}. These values determine the minimum requested temperature of 2ºC and maximum requested temperature of 8ºC of the immunobiologicals.

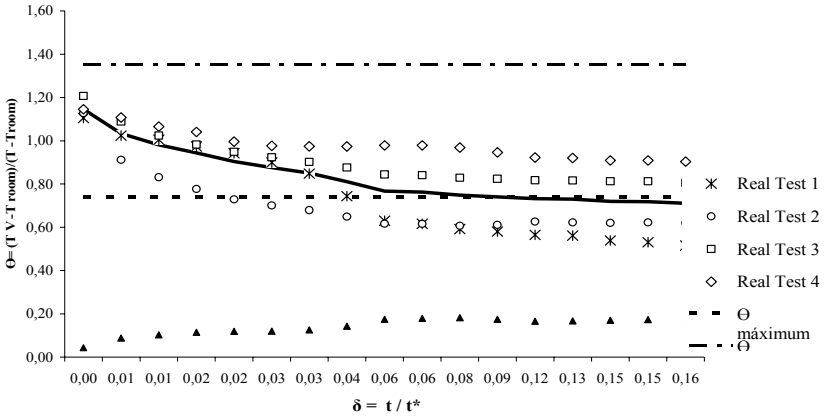

Figure 3. Average temperature in the Real Test

It can be observed on Tab. 2 in Real Test 3 that the maximum time of stability of the immunobiologicals on the requested temperature was 50 minutes.

Table 2. Real Test Data.

	Real Test 1	Real Test 2	Real Test 3	Real Test 4
Room Temperature (º C).	24	23	24	22
Initial Vaccine Temperature - T_V (º C).	6	6	5	7
Maximum Time (minutes) for maintenance of the immunobiologicals on the requested temperature	20	10	50	45

3.2. Results of the experimental tests – Test 1

The mean temperature profile obtained is inside the tolerance band indicated by $\theta_{maximum}$ and $\theta_{minimum}$. As a result, the vaccine was conserved for a much longer period of time than the expected, as show in Tab.3.

The conservation time of the vaccine during the experiments, at the interval of 2 to 8º C, was of approximately 17 hours, that is, 2.125 longer than the desirable period of time.

In Test 1, the temperature curve remained unchanged for the values of θ above 1.00, leading to the conclusion that the temperatures of the vaccines stabilized at very low values, below the temperature at the beginning of the process, according to Fig. 4.

Table 3. Mean values of θ obtained in the positions A, B, C and D.

TEST 1 – θ Values				
δ = t / t *	Mean	Median	Mode	Standard Deviation
0.00	1.00	1.00	1.00	0.00
0.25	1.10	1.10	1.11	0.01
0.50	1.10	1.10	1.11	0.01
0.75	1.10	1.10	1.10	0.00
1.00	1.11	1.11	1.11	0.00
1.25	1.11	1.10	1.10	0.01
1.50	1.11	1.11	1.11	0.00
1.75	1.11	1.11	1.10	0.01
2.00	1.11	1.10	1.11	0.01
2.25	1.10	1.10	1.10	0.00

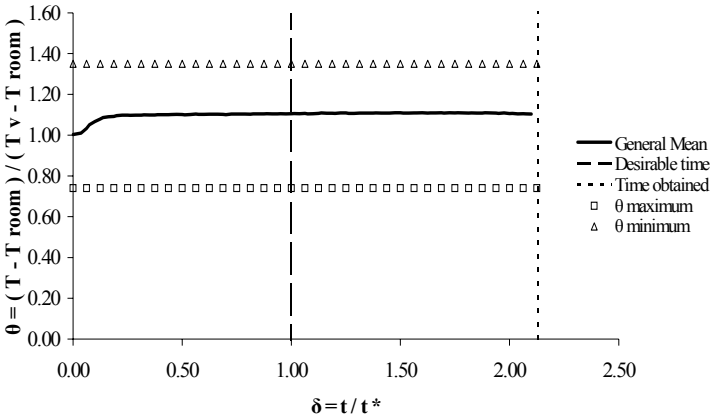

Figure 4. Profile of the mean temperature in the positions A, B, C and D – Test 1

Figure 5 shows the period of time the temperature of the vaccine was maintained as a result of the position of each thermocouple. In position A, due to the vaccine's closeness to the outer wall, its conservation time was shorter when compared to the other positions, which indicates the evaluation of the behaviour of the container in terms of the conservation time obtained in position A.

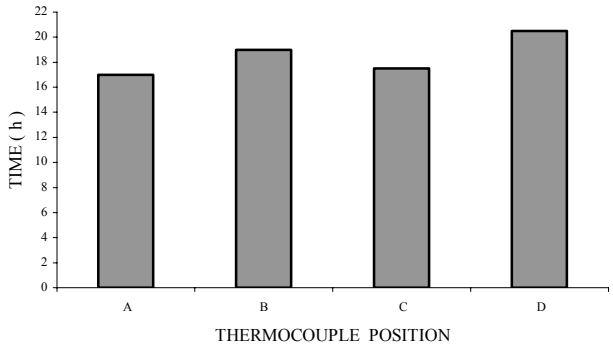

Figure 5. Temperature maintenance time of the vaccine x position of each thermocouple inside the container

3.3. Results of the experimental tests – Test 2

The majority of the vaccination practices occur in the open air in the shade, and the proposed container lived up to the expected performance; with a slight rise in the temperature of the immunobiologicals at the beginning of the process and after the stabilization for the values of θclose to 1.00. It was concluded that the temperatures of the vaccines remained practically the same as those at the beginning of the process as show in Tab.4.

The conservation time of the vaccine at the interval 2 to 8 ° C, obtained during the experiments in this test was of 17.5 hours, that is, 2.1875 times longer than the expected time.

Table 4. Mean values of θ obtained in the positions A, B, C and D.

TEST 2 - θ Values				
δ = t / t *	Mean	Median	Mode	Standard Deviation
0.00	1.00	1.00	1.00	0.00
0.25	1.00	1.01	0.96	0.03
0.50	0.99	1.01	0.95	0.03
0.75	0.99	1.02	0.96	0.03
1.00	1.00	1.03	0.97	0.03
1.25	1.02	1.04	0.99	0.03
1.50	1.03	1.06	1.00	0.03
1.75	1.04	1.06	1.00	0.03
2.00	1.03	1.06	1.00	0.03
2.25	1.02	1.03	1.01	0.02

Figure 6 indicates a very coherent temperature profile, the thermal behaviour being that foreseen for the system, taking into consideration the environment proposed, which contributed to cause a slight rise in the temperature of the system as a whole at the beginning of the monitoring process.

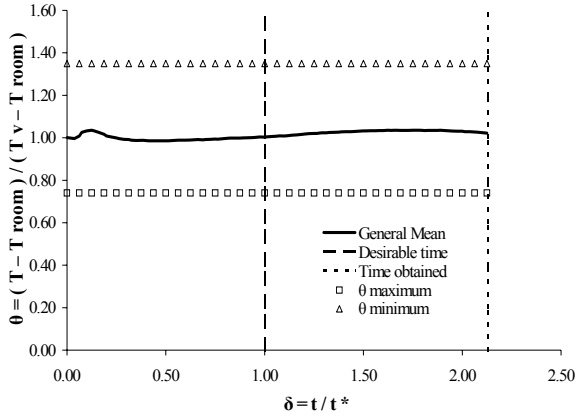

Figure 6. Profile of the mean temperature in the positions A, B, C and D – Test 2

The thermocouple in position A, due to its proximity to the environment outside, presented the shortest conservation periods of time of the vaccine within the temperature band recommended, thus defining the performance of the container in the environment proposed in Test 2.

Figure 7 shows the temperature maintenance time as a result of the position of each thermocouple in the container.

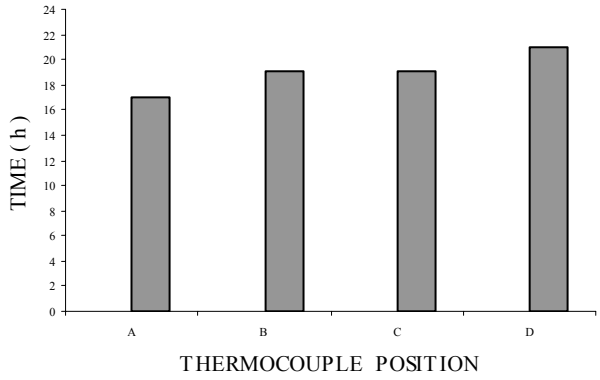

Figure 7. Temperature maintenance time of the vaccine x position of each thermocouple in the container

3.4. Results of the experimental tests – Test 3

The mean temperature profile obtained remained for a long time below what was expected, within the band of tolerance indicated by $\theta_{maximum}$ and $\theta_{minimum}$.

The vaccine's conservation time obtained during the experiments, at the interval 2 to 8º C, was of approximately 2 hours, that is, 0.250 of the desired time.

In the environment proposed, Test 3, the vaccine's conservation times were too short in relation to the desired time, due to the direct incidence of sunlight, which caused the immunobiologicals to heat slightly; as indicated in Figs. 8 and 9, respectively.

Table 5 shows the mean values of θ obtained in positions A, B, C and D.

Table 5. Mean values of θ in positions A, B, C and D – Test 3.

TEST 3 – θ Values				
$\delta = t / t^*$	Mean	Median	Mode	Standard Deviation
0.00	1.00	1.01	1.00	0.01
0.06	0.97	0.98	0.98	0.01
0.13	0.95	0.97	0.97	0.01
0.19	0.93	0.95	0.91	0.02
0.25	0.93	0.96	0.92	0.02

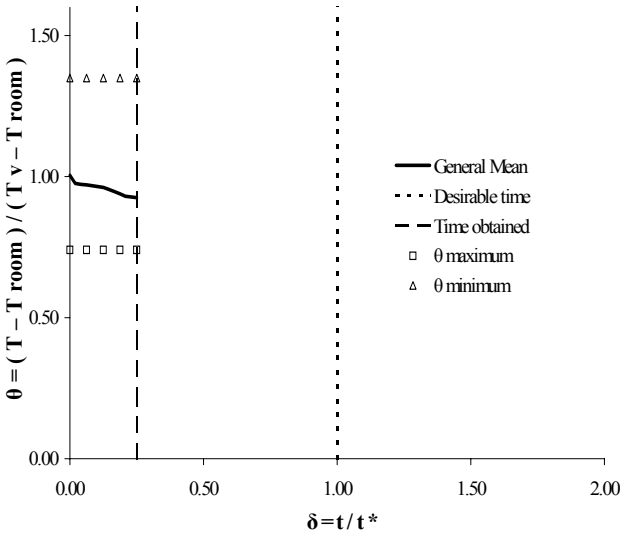

Figure 8. Mean temperature profile in positions A, B, C and D – Test 3

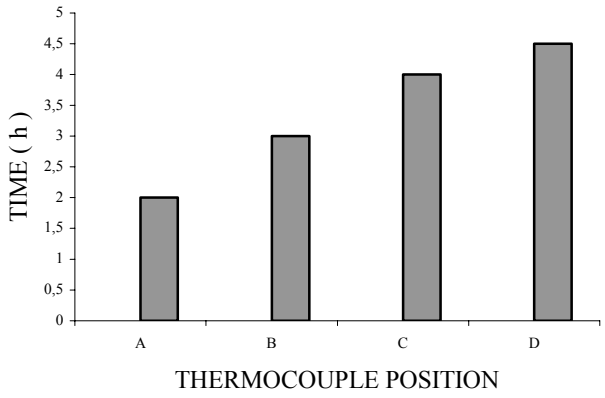

Figure 9. Time of maintenance of the temperature of the vaccine x position of each thermocouple inside the container

3.5. Comparative analyses between Tests 1 and 2

In this section we compared the mean temperature and time obtained from the monitoring of the vaccines in Tests 1 and 2, for which the performance of the container was satisfactory. The purpose of this analysis was to establish a practical and dependable parameter of the mean time for the use of the container, adequate to the temperature band of conservation of the vaccine and desirable time for the maintenance of the dependability of the quality of the immunobiologicals. Table 5 shows the mean values of θ obtained in positions A, B, C and D.

Table 6 shows the mean values of θ obtained in Tests 1 and 2.

The Fig. 10 indicates the mean temperature profile obtained in Tests 1 and 2 with minimum conservation times of 17 and 17.5 hours, respectively, which is 9 and 9.5 hours in excess of the expected time.

Table 6. Mean values of θ in the positions A, B, C and D - Tests 1 and 2.

TESTS 1 and 2 – θ Values				
δ = t / t *	Test 1	Test 2	Mean	Standard Deviation
0.00	1.00	1.00	1.00	0.00
0.25	1.14	1.00	1.07	0.10
0.50	1.14	0.99	1.07	0.11
0.75	1.14	0.99	1.07	0.11
1.00	1.14	1.00	1.07	0.10
1.25	1.15	1.02	1.09	0.09
1.50	1.15	1.03	1.09	0.08
1.75	1.15	1.04	1.10	0.08
2.00	1.15	1.03	1.09	0.08
2.13	1.14	1.02	1.08	0.08

Concluding that the proposed container had a very good response in the environments represented by Tests 1 and 2, they can be used with a very high rate of dependability and performance in either environment.

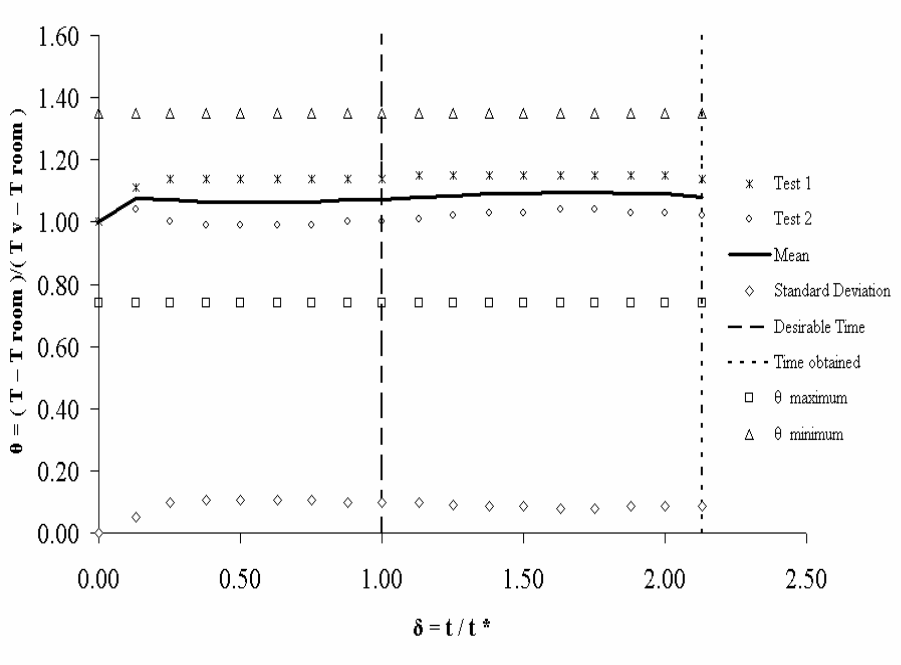

Figure 10. Mean temperature profile Tests 1 and 2

4. CONCLUSIONS

The purpose of this study was to develop a container to be used in the transportation and conservation of immunobiologicals, for a minimum 8 hours in a temperature between 2 and 8 º C.

Based on the Real Test results we can verify that the procedures for immunobiological handling used nowadays on extramural vaccination practices guarantee for a short period of time, about one hour, the immunobiological efficiency.

The differential between the designed container and the existing ones lays the position the vaccines are enclosure in niches, which allows easy handling and provides individual thermal insulation.

After the container was developed, tests were run considering the three environments proposed: Test 1 (in a closed room without refrigeration or incidence of sunlight); Test 2 (in the open air, in the shade) and Test 3 (in the open air with incidence of sunlight).

The results obtained led to the conclusion that in environments without direct incidence of sunlight, besides living up the expectations, the container outdid the performance foreseen concerning desirable conservation time of the immunobiologicals within the temperature band desired. However, in places where the container was exposed to the sunlight, the conservation times were not satisfactory.

In Test 1, the immunobiological remained at a temperature stabilized above the temperature at the beginning of the process, but within the temperature band desired for 17 hours.

As for the second environment proposed, (Test 2), the monitoring process showed that despite the stabilization of the temperatures a little above the rate of stabilization observed in Test 1, the mean time of maintenance of the immunobiologicals within the temperature band required was of 17.5 hours.

In Test 3, as the time obtained was of 2 hours in this environment, heat penetrated mainly through the upper side of the container, which was continuously exposed to direct sunlight, since this side had no thermal walls (barriers).

The conservation time of the container that guarantees the dependability of the immunobiologicals is of 17 hours.

After the conclusion of the project and collection of the experimental results, a patent report was written and submitted to INPI under the provisional registration number 014060000622.

As suggestions for further studies we indicate: Project and study a more effective thermal insulation technique with the inclusion of barriers (walls) in the upper surface of the container, besides a study of a mathematical model capable of simulating adverse temperature and dimension conditions.

5. ACKNOWLEDGEMENTS

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