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Analysis of Cl and Na in Hyperimmune Sera by NAA

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Abstract. The Cl and Na concentration values in four types of hyperimmune sera (*anti-Bothrops, anti- Diphtheria, anti-Rabies and anti-Tetanus*) used for immunological therapy were determined using Neutron Activation Analysis (NAA). These data were compatible with the specifications established by the Word Health Organization (WHO – OMS) and with the Brazilian Official Pharmacopea (Pharmaceutical Code Official of the Country). These data are an important support for quality control of hyperimmune sera production at Butantan Institute (São Paulo city, Brazil), responsible for supplying the Brazilian market.

Keywords: hyperimmune sera, NAA, antivenoms , immunological products, antibodies.
PACS: 82.80

INTRODUCTION

There is a high prevalence of snakes of the genus *Bothrops* in regions of Central and South America. There are more than sixty species identified in these regions, responsible for 80% of snakebites in Brazil, resulting in a critical public health problem for Brazilian population. Also, on lower scale, some infectious agents are also a health problem for the country´s inhabitants. Nowadays, Butantan Institute (São Paulo city, Brazil) meets the Brazilian market´s demand for hyperimmune sera (antivenoms) in its entirety. Sera are stored in ampoules of 5 or 10 mL, depending on the immunological therapy one or more doses can be used by patient during treatment.

These hyperimmune sera are immunological products that contain antibodies used in the treatment of victims of poisonous animals and also of patients with diseases caused by toxins of infectious agents. In the production of hyperimmune sera, several steps are involved: first, horses are immunized with poisonous animals from one or several species (mainly *Bothrops*), or with bacterial toxins and viruses in the case of infectious agents. At the end of each month-long cycle of immunization, a satisfactory period for antibody production, approximately ten liters of blood are collected per house. The final step is the serum treatment: it must be purified in order to diminish the possibility of adverse reactions in patients who will receive the hyperimmune sera (MORAIS et al, 1994)[1]. This treatment implies the accomplishment of a series of biological and chemical tests that assure the quality, effectiveness and safety of the final product. These tests follow the specifications established by the National Health

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Surveillance Agency (ANVISA) document 174 (11Th November, 1996) [2]. The control of pH and proteins are included in these tests as well as the levels of sodium and chlorine in the antivenoms. The presence of Cl and Na is related to the addition of NaCl during the step of sera production.

In this study the *Bothrops* antivenom used for the treatment of victims of poisonous animals, mainly snakebites (responsible for 80% of the cases in Brazil) as well as antivenoms used for the treatment of patients with diseases caused by toxins of infectious agents, such as the causers of *Botulism, Diphtheria*, and *Tetanus*, were investigated using Neutron Activation Analysis for checking chlorine and sodium levels after sera purification. These data can be used for checking the performance of the sera purification and as an important support for its certification and commercialization.

EXPERIMENTAL TESTS

In this investigation four hyperimmune sera (*anti-Bothrops, anti-Diphtheria, anti-Rabies and anti-Tetanus*) produced at Instituto Butantan (São Paulo city) were investigated. For sample preparation aliquots of 100μL (±0.5%) of each serum solution were transferred to filter paper (Whatman n°41). In order to determine the concentration of the elements Na and Cl, the Cadmium Ratio Technique was used for the measurement of thermal and epithermal flux distribution. In this technique, Au foils, both bare and Cd covered (1.5 mm x 1.0 mm), irradiated together with the sample and blank (filter paper) in the IEA-R1 nuclear reactor at IPEN/SP (IEA-R1, 2-4MW, pool type) allowing the simultaneous activation of these materials under the exact same irradiation conditions. This experimental procedure is a routine work for neutron flux determination in the IEA-R1 reactor [3]. All the irradiations were performed in the pneumatic irradiation facility for 30s and counting time of 120s using a HPGe detector (GEM-60195 ORTEC) connected to an ADCAM multichannel analyzer (919E-ORTEC) and to a PC computer. The concentration of each element, was performed using an in-house software (*ATIVAÇÃO*)[4].

RESULTS AND DISCUSSION

Each serum sample and the blank could be irradiated together in function of the half - lives involved, ^{38}Cl ($T_{1/2}$=37 min) and ^{24}Na ($T_{1/2}$=15h).

The qualitative analyses in the blank identified some impurities, such as Al, Ca and Mg but they do not interfere; also Cl and Na were identified but in very low concentration (0.012 – 0.014 gL^{-1} for Na and 0.060 – 0.073 gL^{-1} for Cl).

The concentration values for Na and Cl in hyperimmune sera obtained by NAA were compared with the conventional procedure adopted at Butantan Institute (Titulometry). These data are presented in Table 1 together the limits established by: ANVISA[2] for comparison.

TABLE 1. Na and Cl contents in hyperimmune sera.

Antivenon, Limits [a,b]	**Na, gL^{-1} [2.75 – 3.54]***	**Cl, gL^{-1} [4.25 – 5.45]***
Bothrop		
n=5		
[3.00 – 3.32][a]	**3.16 ± 0.16**	**5.18 ± 0.25**
[2.94 – 3.60][b]	**3.27 ± 0.33**	**5.03 ± 0.50**
Dephtheri		
n=3		
[3.26 – 3.60][a]	**3.43 ± 0.17**	**5.17 ± 0.25**
[2.97 – 3.36][b]	**3.30 ± 0.33**	**5.09 ± 0.50**
Rabies		
n=3		
[3.67 – 4.15][a]	**3.91 ± 0.24**	**5.84 ± 0.39**
[2.94 – 3.60][b]	**3.27 ± 0.33**	**5.63 ± 0.50**
Tetanus		
n=3		
[3.37 – 3.71][a]	**3.54 ± 0.17**	**4.88 ± 0.24**
[3.01 – 3.67][b]	**3.34 ± 0.33**	**5.15 ± 0.51**

* limits established by WHO-OMS together with the Brazilian Pharmacopeia's [2]
[a] limits from NAA for a confidence interval of 68%
[b] limits from Titulometry for a confidence interval of 68%
n: number of samples

According to Table 1 all the results from both techniques agree with the limits established by ANVISA [5]. For Na the values from NAA agree with the Titulometry method in 75% of the cases considering the uncertainty: while anti – *Bothrops* (3.16 ± 0.19) gL^{-1}, anti – *Dephtheri*, (3.43 ± 0.17) gL^{-1} and anti-*Tetanus* (3.54 ± 0.17) gL^{-1} are in agreement with the Titulometry method in confidence interval of 68%, for anti – *Rabies* (3.91 ± 0.24) there is compatibility only considering a confidence interval of 95% (3.43 – 4.39) gL^{-1}. This small variations suggesting that the sodium impurity could be oscillating in the blank. For Cl the agreement between the methods is observed for all the analyses considering a confidence interval of 68%.

CONCLUSION

The concentration for Cl and Na were determined in hyperimmune sera produced at Butantan Institute (SP, Brazil) using NAA technique. The results are in agreement with the Titulometry results as well as with limits established by ANVISA for its certification and commercialization. The NAA data emphasizes the quality control of hyperimmune sera production performed at Butantan Institute.

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