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Mass Spectrometry 2017: Efficacy and Stability of Scorpion Antivenom: At Different Storage Conditions - Elhag DE - UMST University

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Introduction

In many parts of the world the Scorpion bites is an actual public health problem due to the incidence or severity of envenomations is high or for these two reasons at the same time. Many potentially dangerous scorpions inhabit the under developed or developing countries and numerous envenomations go unreported. Officially, it has been estimated that there are 1.2 million scorpion stings per year around the world leading to 3250 deaths (0.27%). The 2014 Annual Report of the American Association of Poison Control Centers 'National Poison Data System (NPDS) reported 16,440 case mentions for scorpion envenomations. However, because of underreporting, this is probably an underestimation of the true number of stings.

Scorpion venoms contain many bioactive components. Several of the long chain peptides have been shown to be responsible for neurotoxic effects. These toxins are basic polypeptides with molecular weights of around 7000 kD, without enzymatic action and have been shown to affect the ion permeability of excitable cells. These preparations are included in the WHO List of Essential Medicines and should be part of any primary health care package where scorpion bites occur.

The antivenom is produced by milking venom from the desired Scorpion, injecting the milked venom into a horse, sheep, rabbit, or goat to stimulate animal immune system. There is an urgent need today to ensure the availability of safe, effective and affordable antivenoms, particularly for those in developing countries, and to improve regulatory control over manufacturing, importation and sale antivenoms.

Scorpion venom contains neurotoxic peptides that interact with ion channels, causing massive damage to the nervous system. Thanks to this interaction, scorpion venom can provoke excitement in the nerves and muscles, hormonal secretion and disturbances in the control of the balance of salt and water and the regulation of blood pressure.

Clinical symptoms during envenomation indicate general stimulation of the autonomic, somatic and peripheral nervous systems. The severity of the poisoning of the scorpion and the increased risk of mortality, especially in children, are mainly attributed to cardiorespiratory pathology.

Materials and Reagents Chemical tests Determination of pH:

The pH of the selected samples was measured using an appropriate pH meter calibrated just before use compared to standard buffer solutions (pH 4 and pH 7).

The WHO obligation to produce antivenom indicates that the pH of the antivenom should be between 6 and 7.

Determination of m-cresol: Preparation of the buffer solution pH 9

Solution A: 6.18 g of boric acid and 7.45 g of potassium chloride (0.1 M) to be dissolved in 1000 ml of water.

Solution B: sodium hydroxide 0.1 N R.

To 1000 ml of solution A, 400 ml of solution B was added, sodium hydroxide was gradually added to the mixture until pH 9 was reached.

Potassium ferricyanide solution

Exactly 5 g of potassium ferricyanide were washed with 3 ml of water and then dissolved and

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diluted with water to 100 ml (this preparation was prepared immediately before use).

Aminoantipyrine R solution: 100 mg of aminoantipyrine were dissolved in 100 ml of pH 9 buffer solution.

Preparation of the sample

Two test groups were prepared. The first group consists of six anti-venom bulbs which were randomly selected from the batch of scorpion antivenom stored in real conditions (3 ± 2 ° C). The second group, composed of six anti-serum ampoules, was subjected to accelerated conditions (25 ± 3 ° C, RH75%). 0.2 ml of each sample (contains up to 0.35% m-cresol) was diluted in 3.5 ml of water to give a final concentration of (200 µg / ml).

Procedure

A total of seven beakers have been labeled for use, the first has been defined as blank. Six more 0.5 ml beakers of the prepared samples were added to each beaker. Then, 5 ml of aminoantipyrine and 5 ml of potassium ferricyanide were successively added to each beaker. The prepared test samples were left for 10 minutes at room temperature, then each sample absorbance was measured at 540 nm using a UV / vis spectrophotometer.

Standard m-cresol preparation

The stock solution of M-cresol solution (250 µg / ml) was prepared by weighing exactly 1 g of m-cresol and transferring it to a 1-liter volumetric flask and the volume was adjusted to the mark using distilled water. Exactly 2.5 ml was taken and made up to 10 ml.

A series of appropriate dilutions were made to construct the standard curve and the concentrations of the samples were determined from the standard curve.

Determination of the total protein content/ determination of Albumin content:

The antivenom solution was diluted with a 25% sucrose solution in 0.01 M phosphate buffer, pH 7, to a protein content of 10 mg / ml. 10 µl samples of the antivenom were applied to the polyacrylamide gel membranes in the separation chamber and subjected to electrophoresis. A standard 1 mg / ml albumin sample was analyzed simultaneously with antivenom serum samples. The gel was then removed from the separation chamber and placed in the development chamber and development was carried out using the same machine according to the following programmed method. The electrophoretic mobilities of antivenom and albumin were compared after staining with Coomassie blue.

Results pH test:

PH measurements of ampoules randomly selected after being subjected to two different storage conditions (real and accelerated) showed that the values were comparable and within the acceptable range indicated for the antivenom produced. WHO's obligation to produce antivenom, said the PH of the antivenom should be between 6 and 7.

Measurements of the m-cresol content of the ampoules were previously stored under appropriate storage conditions (real, 3 ± 2°C) and under accelerated conditions (25 ± 3°C, RH75%). showed that there was a minor change in the concentration of m-cresol, but this change was neither significant nor outside the range of acceptable limits indicated on the ampoule brochure.

Measurement of the protein content of the polyvalent scorpion antivenom randomly obtained from the batch and maintained under the two selected storage conditions, real-time storage condition (3 ± 2°C) and accelerated storage condition (25 ± 3°C, RH75%). showed that the protein content in the two classes of samples was comparable. WHO guidelines state that the total concentration of protein in antivenoms should preferably not exceed 10 g /

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dl, unless a higher protein content is justified and authorized by the competent authority.

Conclusion

The present work has suggested that maintaining the antivenom at the specified temperature and humidity and when exposed to light for a longer period of time does not affect the antivenom in terms of sterility or efficacy.

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