

## عنوان مقاله:

A New Practical Purification Method for Type D Clostridium perfringens Epsilon Toxin by Size-Exclusion (Chromatography (SEC) and Ultrafiltration (UF

## محل انتشار:

فصلنامه طب دامی ایران، دوره 16، شماره 2 (سال: 1401)

تعداد صفحات اصل مقاله: 10

## نویسندگان:

Molarameh Poudineh Morref - *Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

Mohammad Kazem Koohi - *Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

Mojtaba Alimolaei - *Department of Research and Technology, Kerman branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Kerman, Iran*

Tara Emami - *Department of Proteomics and Biochemistry, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*

Jalal Hassan - *Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

## خلاصه مقاله:

**BACKGROUND:** The high potential toxicity of epsilon toxin (Etx) produced by Clostridium perfringens (C. perfringens) type D, has made it the third most lethal clostridial toxin behind botulinum and tetanus, therefore, having a pure and concentrated Etx is very important. **OBJECTIVES:** The aim of this study was to purify Etx as pure as possible with an applicable, cost-effective, multistep purification protocol with the lowest and shortest time. **METHODS:** The purification of the Etx was carried out in multiple consecutive steps; ammonium sulfate precipitation, dialysis, size exclusion chromatography by G<sub>50</sub>, two concentration steps, and ultrafiltration. The Etx activity after different steps was evaluated by the minimum lethal dose (MLD) calculation, according to the standard operating procedure. Toxin quantification was determined using Lowry technique, and its presence and specificity was tracked to identify pure Etx by SDS-PAGE and western blotting. Finally, the purity of Etx was evaluated by capillary electrophoresis. **RESULTS:** The purified Etx formed a single band of about ۳۲.۹ kDa in SDS-PAGE and blotting. The pure Etx concentration was calculated to be ۳.۹ mg/ml and its MLD value was the dilution of ۱/۲۴۰۰۰ after the ultrafiltration step. The presented purification processes to purify Etx resulted in ~ ۸۷-fold concentration and ۸۸.۶% purity. **CONCLUSIONS:** Due to this high Etx purity, the processes used in this study can provide the technical knowledge of toxin production in a larger industrial scale that can be used in development of clostridial toxoid vaccines, as well as quality control and/or diagnostic tests.

## کلمات کلیدی:

Clostridium perfringens, concentration, Epsilon toxin, High purity, purification

