

Technical Report

**A MODIFIED METHOD FOR THE COCENTRATION
AND PURIFICATION OF DIPHTHERIA AND
TETANUS TOXOIDS (A BRIEF REPORT)**

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Key words: DIPHTHERIA/TETANUS/PURIFICATION/TOXOIDS/METHODS

Purification of diphtheria and tetanus toxoids, produced at the Razi Institute, was usually performed by Zinc Chloride precipitation, followed by Ammonium Sulfate fractionation and finally by dialysis of the precipitate in distilled water to remove excess salt present.

Attempts have lately been made to omit the time consuming process of dialysis, and rather desalt the product by gel filtration, through sephadex G - 25 column.

Zinc chloride is first added to the crude toxoid at PH=6/2-6/4 to precipitate the protein which settle after few hours.

The supernate is then discarded and the precipitated proteins are recovered by filter press.

To the semisolid precipitate obtained, is added solid disodium Hydrogen Phosphate to solubilize the precipitate, centrifuged and clear supernatant is collected.

To the concentrated toxoid ammonium sulfate is added, to reprecipitate first the impurities and second to recover the active toxoid fraction, by adding ammonium sulfate to the supernate left from the first precipitation.

Precipitated proteins are then collected by either filtration or centrifugation. Desalting of the product is achieved by passing through sephadex G - 24 column, after which the toxoids are exposed to both in-vitro and in-vivo studies as stated in the pertinent requirement.

RESULTS:

A simple method for the routine production of diphtheria and tetanus toxin is presented. The time of purification by modified technique is significantly decreased which results in a higher production rate.

Table 1 & 2 shows a comparison between average of 5 experimental runs purified by modified method and average of 5 runs purified by routine method.

Table 1: Diphtheria toxoid

Lot No	Original Lf/ml	Purified Lf/ml	Purification Indice P/I	Concentration Indice C/I	Recovery %	Purified Lf/mg P.N	Time of purification(days)
Average of 5 modified runs	60	5000	3.3	83	98	2100	5
Average of 5 Routine runs	70	200	1.95	28	80	1212	8-10

Table 2 : Tetanus toxoid

	Original Lf/ml	Purified Lf/ml	Purification Indice P/I	Concentration Indice C/I	Recovery %	Purified Lf/mg P.N	Time of Purification(days)
Average of 5 modified runs	15	1400	3.75	93	87	597	5
Average of 5 routine runs	20	1250	2.52	62.5	80	565	8-10