AD-786 915

ALLERGENIC AND ANTIGENIC VALUE OF TUBERCULOPROTEIN PREPARED FROM A CULTURE FILTRATE BY THE SODIUM TUNGSTATE METHOD

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Foreign Technology Division
Wright-Patterson Air Force Base, Ohio

20 September 1974

FTD-HC-23-2070-74

FOREIGN TECHNOLOGY DIVISION

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by

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UNCLASSIFIED
Security Classification

AD 786915

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Foreign Technology Division
Air Force Systems Command
U. S. Air Force

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3. REPORT TITLE
ALLERGENIC AND ANTIGENIC VALUE OF TUBERCULOPROTEIN PREPARED FROM A CULTURE FILTRATE BY THE SODIUM TUNGSTATE METHOD

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Translation

5. AUTHOR(S) *(First name, middle initial, last name)*
Ju. P. Kiptilyj and A. O. Jevhlevskyj

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
1967	6 11	9

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	FTD-HC-23-2070-74
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Foreign Technology Division Wright-Patterson AFB, Ohio

13. ABSTRACT
06

11

DD FORM 1 NOV 65 1473

UNCLASSIFIED
Security Classification

EDITED TRANSLATION

FTD-HC-23-2070-74 20 September 1974

BC 7021203

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English pages: 6

Source: Visnyk Silskogospodarskoyi Nauki, Vol. 10, Nr. 2, 1967, pp. 108-112

Country of Origin: USSR
Translated under: F33657-72-D-0853-0005
Requester: FTD/PDTR
Approved for public release; distribution unlimited.

U. S. BOARD ON GEOGRAPHIC NAMES TRANSLITERATION SYSTEM

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А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

*ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ё in Russian, transliterate as yë or ë. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

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GRAPHICS DISCLAIMER

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RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

Russian	English
sin	sin
cos	cos
tg	tan
ctg	cot
sec	sec
cosec	csc
sh	sinh
ch	cosh
th	tanh
cth	coth
sch	sech
csch	csch
arc sin	$\sin^{-1}$
arc cos	$\cos^{-1}$
arc tg	$\tan^{-1}$
arc ctg	$\cot^{-1}$
arc sec	$\sec^{-1}$
arc cosec	$\csc^{-1}$
arc sh	$\sinh^{-1}$
arc ch	$\cosh^{-1}$
arc th	$\tanh^{-1}$
arc cth	$\coth^{-1}$
arc sch	sech^{-1}
arc csch	csch^{-1}
rot	curl
lg	log

ALLERGENIC AND ANTIGENIC VALUE OF TUBERCULOPROTEIN PREPARED FROM A CULTURE FILTRATE BY THE SODIUM TUNGSTATE METHOD*

Ju.P. Kiptilyj** and A. O. Jevhlevskyj***

Perfecting tuberculosis diagnostic methods is one of the contemporary problems in the fight against these illnesses. Until now, the allergenic method of diagnosis was widely applied as one of the most sensitive methods in detecting this sickness. During recent years, concurrently with allergenic diagnosis, researchers have paid more and more attention to the serologic reactions, with the help of which tuberculosis antibodies in the blood serum of animals that are infected with tuberculosis can be revealed.

The complement fixation reaction (RZK) in the case of tuberculosis was first utilized in 1901, but serologic methods were not widely applied in practice, and were insufficiently developed.

In the works of the authors [1 - 9, et al.], it was proved that in cases of unclear and disputed diagnoses of tuberculosis, the RZK,

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*This work was done under the leadership of Professor V. I. Rotov and Professor N. N. Ivanov.

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the prolonged complement fixation reaction (RTZK), hemoglobinometry (RHA), hemolysis (RH) and diffusive precipitation (RDP) could help the clinician.

The diagnostic value of allergenic and serologic reactions depends on the quality of the allergens and antigens involved. Thus, one of the more important elements in allergenic and serologic diagnosis of tuberculosis is to isolate and establish the standard complete antigens and allergens.

The tuberculosis proteins that are produced in synthetic media, as compared to Old tuberculin, are better for diagnosis, because they do not have protein derivatives from the media or any other extraneous substances.

In the culture media, where the tuberculosis microbacteria are grown to produce tuberculosis proteins (in the media: Long, Soton, Dorset, Lind III, UNDEV 4, Linikova), the source of nitrogen is asparagin and glycocoll. These media are expensive; moreover, there is often observed an unevenness in the growth of tuberculosis microbacteria cultures, and only a small quantity of bacterial masses and tuberculosis proteins is produced. For that reason, we conducted experiments in 1963 - 1964 to study how to replace the expensive components of the med'a in order to make the process more economically feasible, and guarantee a stable and satisfactory growth of tuberculosis bacteria. In this regard, we studied organic acids of di- and tri-carbon cycles (oxalo-acetone, tartaric acid, amberic, nitrotartaric, citric acid and malic acid), amophose, diamophose, superphosphate, urea, ammonium sulphate and other chemical compounds.

Satisfactory results in increasing the bacterial mass and the quantity of protein in the culture filtrate were achieved in the synthetic media by using citric acid.

In order to prepare this antigen, five strains of commercial microbacteria (3 of oxen and 2 of humans) obtained from Kurak Biofactory were seeded on the synthetic media with citric acid. The

cultivation in one thermostat lasted two months, at a temperature of 37 - 38°.

From the two-month old cultured filtrates, a dry, pure tuberculosis protein was produced by precipitation of the active initial filtrate by a 5% solution of sodium tungstate, followed by succeeding reprecipitation with a half-saturated solution of sodium sulphate. The residue obtained was dialized, poured into vials and exposed to lyophilic drying. The yield of tuberculosis protein was 2 - 3 times greater than when produced by another method at the biofactory.

The amount of tubercular units (T.U.) in 1 mg/ml was tested in the researched tuberculosis protein on sensitized guinea pigs, and was compared with PPD (purified protein derivative), prepared by the method of the Ukrainian Research Institute of Experimental Veterinary Medicine (UNDIEV). The sensitization was carried out by a single introduction under the skin of the guinea pigs of a 10 mg culture of BCŽn in one ml of physiological solution. It took effect 30 - 40 days after infection.

The results of titration of PPD are shown in the table.

Thus the coefficient of activity of the researched tuberculin is equal to 1.05, and 1 mg/ml contained 52,800 T.U.

Instead of the standard preparation for the titration of tuberculoprotein, which was prepared with sodium tungstate, we took the tuberculoprotein prepared by the UNDIEV method and the Leningrad Institute of Vaccines and Serums, which earlier were titrated and compared to the standard Copenhagen PPD, with a known quantity of tubercular units (50,000) in 1 mg/ml.

The titration results of the researched antigens on the sensitized guinea pigs showed their allergenic activity. The antigen characteristics of tuberculoproteins, prepared by precipitation of the active raw material of culture filtrate, prepared with sodium

RESULTS OF TITRATION OF PPD PRODUCED BY THE METHOD OF UNDIEV AND BY PRECIPITATING THE ORIGINAL CULTURE WITH SODIUM TUNGSTATE

No. of guinea pig	Nature of reaction (in hour)	PPD, series No. 7, Method: UNDIEV				PPD, series No. 7a, sodium tungstate			
		1:80	1:160	1:320	1:640	1:100	1:500	1:1000	1:2000
1	24	$\frac{19+19}{2}=19$	$\frac{11+11}{2}=11$	$\frac{10+10}{2}=10$	$\frac{9+9}{2}=9$	$\frac{20+20}{2}=20$	$\frac{14+14}{2}=14$	$\frac{11+11}{2}=11$	$\frac{9+9}{2}=9$
2	24	$\frac{17+17}{2}=17$	$\frac{13+13}{2}=13$	$\frac{10+10}{2}=10$	$\frac{8+8}{2}=8$	$\frac{18+18}{2}=18$	$\frac{12+14}{2}=13$	$\frac{12+10}{2}=11$	$\frac{9+9}{2}=9$
3	24	$\frac{20+20}{2}=20$	$\frac{15+15}{2}=15$	$\frac{11+11}{2}=11$	$\frac{8+8}{2}=8$	$\frac{21+21}{2}=21$	$\frac{16+14}{2}=15$	$\frac{11+11}{2}=11$	$\frac{9+9}{2}=9$
4	24	$\frac{16+16}{2}=16$	$\frac{11+11}{2}=11$	$\frac{9+9}{2}=9$	$\frac{7+7}{2}=7$	$\frac{17+17}{2}=17$	$\frac{11+13}{2}=12$	$\frac{9+11}{2}=10$	$\frac{8+8}{2}=8$
5	24	$\frac{18+18}{2}=18$	$\frac{12+12}{2}=12$	$\frac{9+9}{2}=9$	$\frac{8+8}{2}=8$	$\frac{19+19}{2}=19$	$\frac{13+11}{2}=12$	$\frac{9+9}{2}=9$	$\frac{8+8}{2}=8$
6	24	$\frac{21+21}{2}=21$	$\frac{16+16}{2}=16$	$\frac{11+11}{2}=11$	$\frac{9+9}{2}=9$	$\frac{22+22}{2}=22$	$\frac{16+16}{2}=16$	$\frac{12+12}{2}=12$	$\frac{10+10}{2}=10$
Avg. data on create dimension		111 : 6=18,5	81 : 6=13,5	60 : 6=10	49 : 6=8,1	117 : 6=19,5	82:6 – 13,6	64 : 6=10,7	53 : 6=9
Sum of avg. values of No. of skin create dimensions (in mm)		18,5+13,5 +10 +8,1 =50,1				19,5+13,6 +10,7+9 =52,8			

Coefficient of activity $K = \frac{52,8}{50,1} = 1,05$

tungstate were studied by us using the RHA method according to Middlbrook and Dubos, and the RH method according to Middlbrook and Fisher.

We studied the blood sera (62 tests) of grown cattle who reacted positively to the intradermal introduction of tuberculin; for purposes of control, we employed 60 samples of blood serum from healthy cows, negatively reacting to tuberculin, from farms unaffected by tuberculosis.

In order to perform the RHA and RH, we took fresh erythrocytes from sheep, and sensitized them with the researched antigen in the amount of 2 ml of tuberculoprotein per 0.1 ml of erythrocytes that were washed 3 times in physiological solution. We kept the solution obtained in a thermostat at a temperature of 37° for two hours, periodically mixing it every 10 - 15 minutes. Then we washed the sensitized erythrocytes three times with physiological solution in a centrifuge at 1500 rev/min, and again remixed it with 50 ml of physiological solution. We used the obtained 0.2% suspension of erythrocytes for the RHA and RH.

We applied the following RHA method: After inactivation, adsorption of the heterogeneous hemagglutinins and hemolysins, and after preparation of the corresponding serum dilutions (volume 0.5 ml, dilutions 1 : 8 — 1 : 256), we administered 0.4 ml of 0.2% suspension of sensitized erythrocytes under the skin. The samples were collected and placed in a thermostat for two hours at a temperature of 37°, then at room temperature for 18 - 24 hours, after which we studied the reactions.

The RH test was carried out in an analogous manner. The effective dose of the complement equaled 0.05 ml of the dry complement of the guinea pig, dissolved with physiological solution (1 : 3), which was twice adsorbed by the sheep-erythrocyte precipitate — i.e., 15 parts of complement to one part of erythrocytes.

Positive results in serum dilutions of 1 : 32 were considered as the diagnostic titer for both reactions.

During the serological research of blood serum (from 62 animals, positively reacting to the intradermal introduction of tuberculin) 46 reacted positively to RHA, one doubtfully and fifteen negatively; and to the RH-49 — 1 and 12, respectively.

During the study of the specific characteristics of the reactions, 60 blood samples, taken from healthy cattle that reacted negatively to tuberculin, were tested. For RHA there were 2, for RH there were three tests, which gave positive results in a titrate of 1 : 32.

The research conducted allows us to conclude that tubercular proteins, prepared by precipitation of active initial batches from the culture filtrate by the use of sodium tungstate have a high allergenic effect on sensitized guinea pigs. The tuberculosis protein, prepared by a suitable method, is actively absorbed in the erythrocytes of sheep blood and can be used as an antigen for RHA and RH in the diagnosis of tuberculosis.

REFERENCES

1 [illegible]

2 [illegible]

3 [illegible]

4 [illegible]

5 [illegible]

6 [illegible]

7 [illegible]

8 [illegible]

9 Wadsworth C. Dubos R. J. Specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli. J. exper. med. 1948, v. 88 pp. 521–526.