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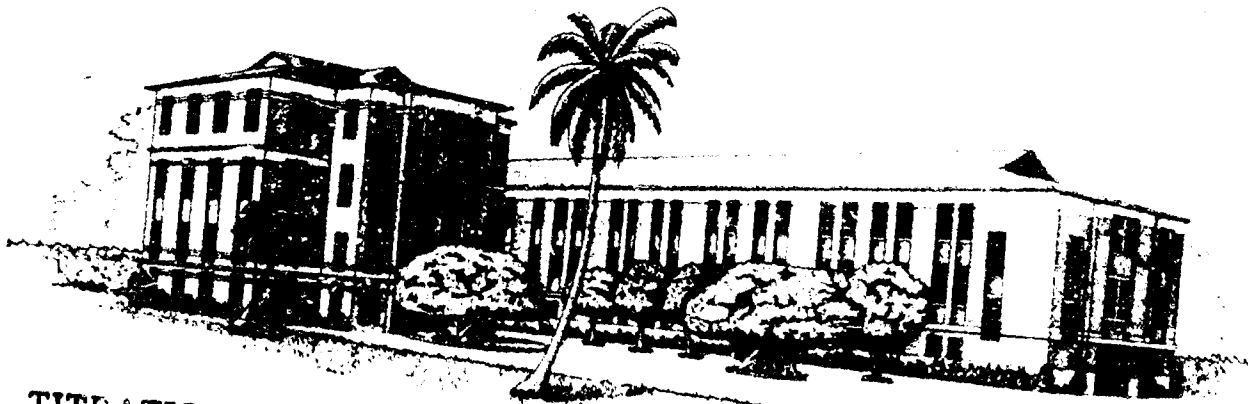
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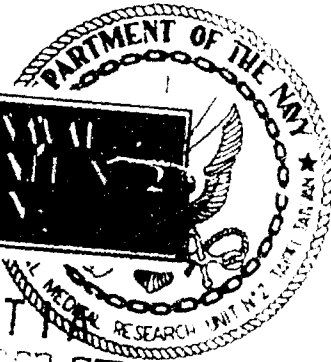


TITRATION OF SMALLPOX VACCINES FROM TEN COUNTRIES SENT
TO EAST PAKISTAN DURING THE 1958 SMALLPOX EPIDEMIC

NM 52 11 02.4.1

18 August 1958

UNITED STATES VETERINARY
MEDICAL RESEARCH CENTER
FEDERAL BUREAU OF INVESTIGATION



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SUMMARY

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(A)

NAVAL MEDICAL RESEARCH UNIT NO. 2

APO 63, SAN FRANCISCO, CALIFORNIA

18 August 1958

**TITRATION OF SMALLPOX VACCINES FROM TEN COUNTRIES SENT
TO EAST PAKISTAN DURING THE 1958 SMALLPOX EPIDEMIC***

By

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**Research Project Report NM 52 11 02.4.1, the Bureau of Medicine
and Surgery, Navy Department, Washington, D. C.**

***From the United States Naval Medical Research Unit No. 2
(NAMRU-2), Taipei, Taiwan (Formosa) and the Department of Medi-
cine, The School of Medicine, University of Chicago, Chicago, Ill.
This investigation was supported in part by a contract Nonr-2121(07)
between the Office of Naval Research and the University of Chicago.**

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**The opinions and assertions contained herein are those of the
authors and are not to be construed as official or reflecting the views
of the Navy Department or the Naval Service at large.**

SUMMARY

Smallpox vaccines of 14 different manufacturer's from 10 countries were titrated for infectivity on chick embryo chorio-allantoic membranes. Samples of all these vaccines were collected in Dacca, East Pakistan during May 1958. With one exception the vaccines showed high enough infectivity titrations to be considered of adequate potency. Because of the lack of control of the conditions to which the vaccines were subjected prior to infectivity titrations, no conclusion can be drawn from these tests concerning the superiority of any manufacturer's vaccine or any type of vaccine. Within the limitation of the present investigation, a favourable attitude can be taken in emergency situations toward the use of smallpox vaccine produced in a variety of laboratories and shipped by modern methods to remote parts of the world.

During the 1958 smallpox and cholera epidemics in Pakistan, the United States Naval Medical Research Unit No. 2 sent a team of scientists to East Pakistan to advise and assist in laboratory diagnosis and vaccine production. Many countries of the world had sent smallpox vaccine to aid in the control program undertaken by the Pakistan government. Samples of many of these vaccines from the various lots received were made available to us during May 1958 by the Pakistan government for potency testing. This report is for the purpose of showing that despite the varied methods of shipping and handling from all over the world the great majority of these vaccines of widely different manufacture showed adequate potency.

MATERIALS AND METHODS

The vaccines that were titrated were collected in Dacca, East Pakistan, presumably from recently received lots. All had been removed from shipping containers and each lot sampled was being used in the field. A few of the samples tested had been taken into the field and returned to Dacca. Samples were not obtained from all countries shipping vaccine to Pakistan. Some of the samples from partially used boxes or returned from field use did not have identifying numbers and expiration dates. A list of the vaccine samples tested with the information available follows:

- 1) Lederle Laboratories Division, American Cyanamid Co. New York City, N. Y. Chick Embryo vaccine, lyophilized, diluent provided. Lot No. 7-1146-53A. Diluent No. 8441-191B. No expiration date. 2 ml per vial, 100 vaccinations per vial.
- 2) Wyeth Laboratories, Inc. Marietta, Pennsylvania, U. S. A. Lymph vaccine, glycerinated. Lot No. 124907. Expiration date Aug. 9, 1958. One dose per capillary, 10 tubes per pack.
- 3) National Drug Co., Philadelphia, Pennsylvania, U. S. A. Lymph vaccine, glycerinated. Lot number and expiration date unknown. One dose per capillary.

- 4) Lister Institute of Preventive Medicine, Elstree, Herts, England. Lymph vaccine, lyophilized, diluent provided. Batch No. 6687. Date manufacture: 4-11-58. 25 doses per tube.
- 5) Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada. Lymph vaccine, glycerinated. Lot 601-1. Code: Melon. Expiration date 8-9-58. 1 ml per vial.
- 6) Commonwealth Serum Laboratories, Melbourne, Australia. Lymph vaccine in 50% glycerine. Batch No. 90671-0070. Date of issue 4-23-58. 100 doses per vial.
- 7) Landes-Impfanstalt, Haroldstrobe 17, Dusseldorf, Germany. Lymph vaccine, in glycerine. Ly-Nr. 131. Versanbuch-Nr. B392. Expiration date 5-21-58. 1000 doses per 4 tubes.
- 8) Behring Werke, Marburg-Lahn, Germany. Lymph vaccine, lyophilized, diluent provided. Op - Nr. 558. Date of preparation 5-5-58. E. 50/51/E197. 100 doses per vial.
- 9) Serum and Vaccine Institute, Berne, Switzerland. Lymph vaccine, in glycerine. No lot number. Expiration date August 20, 1958. 100 doses per vial.
- 10) Gamaliya Institute, 182 Shokinskaya Street 33, Moscow, Russia. Lymph vaccine, lyophilized, diluent provided. Serial No. 104-1, Kohtp 4492. Expiration date December 24, 1958. 10 doses per ampoule.
- 11) Kitasato Institute for Infectious Diseases, Tokyo, Japan. Lymph vaccine, in glycerine. Lot#173. Date of issue 5-21-58. Expiration date 7-20-58. 10 doses per capillary.
- 12) Department of Health, Manila, Philippines. Manufacture unknown. Lymph vaccine in dried state, diluent provided. Identifying number and expiration date unknown. Recommended doses per vial unknown.
- 13) Pasteur Institute, Tehran, Iran. Lymph vaccine, in glycerine. Identifying number and expiration date unknown. One dose per capillary tube.

- 14) Institute of Public Health, Dacca, East Pakistan. Lymph vaccine in glycerine, No. 61F. Production Date 3-5-58. 2 ml per vial, 100 doses.

Vaccines Nos. 3, 12 and 13 were received without identifying labels. They are presumed to be from the stated source on the basis of a handwritten note accompanying the material.

All vaccines were transported under refrigeration to Taipei where the titrations were carried out. The chorio-allantoic membrane (CAM) of the chick embryo was employed for titration of the vaccines. The method used was that of Burnet of counting the pock lesions produced on the membrane. Studies of this method have been made recently by Overman and Tamm, and Cockburn et al. Briefly the following procedure was followed.

Dried vaccines were reconstituted according to the instructions of the manufacturer. The liquid form of vaccinia suspension was then diluted as follows: 0.1 ml of each vaccine was mixed with 9.9 ml of diluent to give a 10^{-2} dilution and ten fold serial dilutions made through 10^{-7} . The diluent used was McIlvaine's 0.01 M phosphate buffer (pH:7.4) containing 10% broth, 50 units per ml of penicillin and 2 mg per ml of streptomycin. 0.1 ml of each dilution (10^{-3} through 10^{-7}) was inoculated on the CAM of each of five 11 - 12 day old eggs. After inoculation, eggs were incubated at 35°C for 44-48 hours and opened to record the number of pocks on the CAM. The potency is expressed in infectious units per ml of the original vaccinia suspension. This is computed from the mean pock count at the lowest dilution giving discrete, countable pocks.

RESULTS

The results of the infectivity titrations of the 14 samples of vaccine are shown in the accompanying table. All the vaccines were titrated at one time and repeat titrations were performed on the three vaccines with the lowest infectivity titre along with two high titre vaccines. The repeatability of the titrations on second test was within one dilution tube which represented less than a 10 fold variation from the first test.

The studies of Cockburn and coworkers has suggested that vaccines which show pock count infectivity titration of 10 million units per ml. will prove to be of excellent potency as measured by the percentage of vaccination "takes" produced in susceptible individuals. * The table shows that 11 of 14 vaccines titred to greater than 10 million infectious units per ml. Vaccine No. 10 showed nearly 10 million units and the No. 7 vaccine on retest also approached this number. Only vaccine sample No. 12 fell significantly below the optional 10 million units. Since this vaccine had been removed from its original carton and was unlabeled there was no way to check how long it had been in Pakistan or the conditions of shipping and storage. Also no expiration date was known.

No consistent difference in potency was found between the lyophilized and glycerinated vaccines. The one vaccine of chick embryo origin (Lederle) showed high titre infectivity.

* These workers found a theoretical relation of 99% "takes" with vaccine showing pock count infectivity of 1.3×10^7 and 90% "takes" with vaccines showing 2.4×10^6 infectivity units.

Table. Infectivity titre of 14 smallpox vaccines tested on chorio-allantoic membrane of chick embryos.

<u>VACCINE SOURCE & TYPE</u>	<u>Day of test</u>	<u>INFECTIOUS UNITS PER ML</u>	
		<u>6-23-58</u>	<u>7-1-58</u>
1) USA, Lederle, (Chick Embryo) lyophilized		2.6x10 ⁷	2.2x10 ⁷
2) USA, Wyeth, glycerinated		3.3x10 ⁷	
3) USA, National Drug, glycerinated		6.0x10 ⁷	
4) England, Lister, lyophilized		3.3x10 ⁷	1.1x10 ⁸
5) Canada, Connaught, glycerinated		2.2x10 ⁷	
6) Australia, Commonwealth, glycerinated		2.8x10 ⁷	
7) Germany, Landes Impfanstalt, glycerinated		1.0x10 ⁶	7.6x10 ⁶
8) Germany, Behring Werke, lyophilized		1.3x10 ⁷	
9) Swiss, Serum Institute, glycerinated		1.8x10 ⁷	
10) Russia, Gamaliya, lyophilized		9.0x10 ⁶	9.4x10 ⁶
11) Japan, Khasato, glycerinated		6.4x10 ⁷	
12) Philippine, Dept. of Health, dried		4.6x10 ⁵	1.6x10 ⁵
13) Iran, Pasteur, glycerinated		3.1x10 ⁷	
14) Pakistan, Dacca, glycerinated		1.2x10 ⁷	

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