EVALUATION OF POTENCY OF MARKETTED SMALLPOX VACCINES

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(1) Potency of various smallpox vaccines tested:

When an epidemic of smallpox occurred in early 1946, several specimens of the marketted smallpox vaccine reached us through Ministry of Health and Social Affairs, and these were evaluated as regards their potency by means of intradermal inoculation in rabbits as the preparatory tests for the National assay to come. Test techniques employed were as follows:—

0.5 cc of vaccine lymph under test is suspended in 4.5 cc of sterile physiological saline solution of pH 7.6 to 7.8, well shaken, centrifuged at 3,000 r.p.m. for 15 minutes, and then the supernatant is diluted with sterile saline solution into a set of 1/10 sequent series up to 1/10,000,000 dilutions.

Three rabbits with hair clipped off are injected intradermally with four kinds of above dilution, 0.2 cc each along the scapular line 2 cm apart, the highest dilution being injected nearest to the neck and the other three downwards; each rabbit getting a set of 1:10,000,000, 1:1,000,000, 1:100,000 and 1:10,000 dilutions. Reaction is read on two occasions, on the 6th (24~5 hrs.) and the 7th (24~6 hrs.) days after injections, and an eruption measuring 8 mm or larger in diameter is judged as a positive take. The number of the highest dilution causing a positive reaction is termed the eruptive unit of the vaccine lymph tested.

A potent vaccine lymph (standard vaccine lymph of known eruptibility) is inoculated at the same time, on the other side of the dorsal skin of the animals to control the individual differences in susceptibility to the virus.

Table 1. Evaluation of smallpox vaccine prepared by several manufacturers.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard lymph</td>
<td>10,000,000</td>
</tr>
<tr>
<td>No. 1</td>
<td>1,000,000 (a little over)</td>
</tr>
<tr>
<td>No. 2</td>
<td>1,000,000</td>
</tr>
<tr>
<td>No. 3</td>
<td>1,000,000</td>
</tr>
<tr>
<td>No. 4</td>
<td>1,000,000</td>
</tr>
<tr>
<td>No. 5</td>
<td>100,000</td>
</tr>
<tr>
<td>No. 6</td>
<td>100,000</td>
</tr>
<tr>
<td>No. 7</td>
<td>10,000 (100,000)</td>
</tr>
<tr>
<td>No. 8</td>
<td>10,000</td>
</tr>
</tbody>
</table>

As seen in Table 1, the present experiment disclosed an outstanding discrepancy among the smallpox vaccines obtained from various sources. In effect, the titres varied from 10,000 to 10,000,000.
(2) Observations on the durability of the above smallpox vaccines:

A series of preservation experiments was carried out under the room temperature during from March to the end of April. The average room temperature under which the lymphs were preserved was 7°C (Max. 11.9°C, Min. 2.2°C) in March and 12.7°C (Max. 17.5°C, Min. 8.1°C) in April. Periods for preservation in the present experiment date back to the date of manufacture, presumably the date of issue of each sample.

The outcome is seen below in graphic form:

As may be clearly seen in the graph, the standard lymph which has a titre of 1:10 millions, maintained its original titre during 40 days under room temperature of from March 10 to April 20. It, however, dropped to a million first after 50 days.

No. 1 lymph which showed a titre of a million and a fraction upon test after 30 days was found to be 1:100,000 on the 40th to 50th days.

No. 3 lymph, known to be 1:1,000,000 units was found keeping its original titre, during 20 days and dropped to 1:100,000 units after further 10 days.

No. 5 lymph, which kept its original 1:100,000 titres for 20 days, dropped to 1:10,000 after further 10 days.

No. 6 lymph which proved to be 1:100,000 when tested after 10 days from the date of its manufacture, showing the gradual drop, proved to be 10,000 after 20 days, and 1,000 after 40 days. With all the above, we could accept the old view, often expressed by competent workers engaged in the lymph manufacturing, that the potent lymph is very well preservable for a long period of time. Moreover, the present experiment has taught the fact that the specimens which are not sufficiently potent and the specimens, which seem to be highly diluted, is very liable to undergo the loss of potency.

![Graph showing variation in potency of various smallpox vaccines.](image-url)
(3) An evaluation of the above lymphs by vaccination on man:

The outcome of the above tests by the animal experiment coincided with the results of the vaccination on man as follows:

1) An example of a practice upon vaccinated adults:

With the standard lymph of 10 million titres, 22 out of 36 revaccinated personnels, were successfully vaccinated, that is 61.11%. With No. 6 lymph, 100,000 in titre, only 32 among 114 revaccinated personnels, showed "successful take", that is only 28.9%.

2) Another example, conducted upon vaccinated adults:

With the standard lymph 92.85% "take" cases (13 out of 14 personnels), and with No. 6 lymph only 46.3% "take" (68 of 149 personnels) were caused in this experiment.

These two experiments were conducted early in March.

3) Outcome in children under 8 years old (March, 24):

As seen above, the revaccination performed following the primary vaccination with a guaranteed lymph, seems to cause "take" in a good many instances after 2 years, and in 100% after 4 years.

4) An example of primary vaccination:

This is the experiment carried out in Niigata area, in which, primary vaccination executed upon infants with No. 8 lymph (10,000 units) resulted in 23 "take" in 31 infants, namely in only 72%.

In the control, performed with No. 3 lymph (a million units), 13 out of 13, namely, 100% positive reactions were caused.

5) An experience at Toshima-Infectious Disease-Hospital:

Eighty personnels, in whom vaccination failed to take only a month ago, were vaccinated again with a potent lymph, known to be of 10 million units, and 18 personnels, namely, one-fourth were found to be "take".

According to the personal communication from Dr. M. Uchida, the head physician of the hospital, a definite number of contract cases have been found among the men of the hospital, who had received the revaccination quite recently. Hence, it is very noteworthy that there occurred no contract cases for the first time following the said revaccination by means of a very potent lymph.

The finding seems to be very significant, because it confirms the contention of experienced workers, that the use of a lymph, as highly potent as possible is absolutely necessary in the case of revaccination, and it strongly suggests the probability of complete exemption from contraction even of the personnels, who have a close contact daily
with smallpox patients. With these evidences, we reached the conclusion that the smallpox vaccine for general use ought to have a titre of a million at least.

Presumably, hereafter, the issue of lymph with titre under a million will not be authorized for release as a perfect lymph available for every instance. One-tenth million titre lymph may be marketted for the primary vaccination only. Ten thousand titre lymph should be discarded at all events.

SUMMARY

(1) Eight specimens of marketted vaccine lymph were tested as to their potency by intradermal inoculation upon rabbits, and an outstanding divergency in their potency, such as 10 thousands to 10 millions of intradermal unit was detected.

(2) Upon test on the durability of potency of lymphs, kept under room temperature during March and April, average temperatures for the months being 7°C, and 12.7°C respectively, it was evidenced that a potent lymph could be preserved in its original virulence for a very long time, but a lymph with low titre lost its virulence in a very short period.

(3) The potency, as estimated by the intradermal inoculation upon rabbits, was seen to well coincide with the rate of successful vaccination on human being, executed by the ordinary scarification method. The minimum requirement for the potency of smallpox vaccine for general use seems to be one-million intradermal units.