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Studies On Laboratory Testing Of Smallpox Vaccines Used In India Under The National Smallpox Eradication Programme

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Moisture content of 1508 batches of freezedried smallpox vaccine, imported and indigenous, was determined. There exists a notable variation in moisture content of batches manufactured at different centres. The vaccine batches from all the production centres never contained moisture over 4%. However, variation in moisture content below this level seems to have no relationship with the stability of the vaccine.

Introduction

There is very little published work regarding the determination of moisture content of freezedried smallpox vaccine. Even the expert group of W.H.O. on requirements for biological substances (1959, 1966) have not recommended any permissible standard of the percentage of residual moisture in the freezedried product. Nomura **et al** (1965) determined the residual moisture content of the freezedried smallpox vaccine by using Abderhalden's apparatus and recommended that the moisture content should not be over three per cent. This has been arbitrarily adopted in the Minimum Requirements for Biological Products, Ministry of Health and Welfare, Japanese Government, 1965. Westwood (1968) is of the view that the vaccinia virus may be freezedried without loss of titre, provided that the final two per cent residual moisture is not removed.

The main object of this study was to determine the moisture content of the freeze-dried smallpox vaccine, imported (USSR) and produced indigenously, and to correlate it with the stability of the vaccine at (*i*) boiling temperature for one hour and (*ii*) 37°C for 28 days.

Material and Methods

Pyrex glass weighing bottles and their glass stoppers were thoroughly washed and dried in hot air oven at 100°C for 2 h and kept in the desiccator over concentrated sulphuric acid for half an hour. The bottles were then weighed up to fifth decimal place on a semi-automatic electric balance.

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Contents of ten ampoules of freezedried smallpox vaccine batch were pooled in the previously weighed bottle and weighed. The bottles were then kept in the hot air oven at 100°C, stoppers removed, vaccine exposed to 100°C for one hour. They were kept in the desiccator over concentrated sulphuric acid for half an hour for cooling. The bottles were weighed again and weight of the anhydrous vaccine obtained. The percentage of moisture content was then calculated.

Heat resistance test on dried vaccine

37°C for 28 days : Five ampoules of USSR and four ampoules of each batch of Indian freezedried smallpox vaccine after keeping at 37°C for 28 days were tested for potency on the chorio-allantoic membrane of 12-day old chick embryo according to the technique employed earlier (Sehgal **et al** 1969).

Rapid stability, viz. boiling test : Five ampoules of USSR and four ampoules of each batch of indigenous freezedried smallpox vaccine after keeping in boiling water for one hour were tested for potency on the chorio-allantoic membrane of 12-day old chick embryo.

Results

One thousand five hundred and eight batches of freezedried smallpox vaccine, imported and indigenous, were subjected to moisture content determination test. The results are set out in Table I.

Table I. Number of batches of freezedried smallpox vaccine by moisture content

Origin of vaccine	Moisture percentage				Total <4%
	<1	1-1·9	2-2·9	3-3·9	
USSR State Vaccine Institute, Patwadangar	15 (8·67%)	114 (65·89%)	42 (24·27%)	2 (1·15%)	173
King Institute of Preventive Medicine, Guindy, Madras	171 (31·03%)	316 (57·35%)	62 (11·25%)	2 (0·36%)	551
Vaccine Institute, Belgaum	115 (52·27%)	92 (41·81%)	12 (5·45%)	1 (0·45%)	220
Institute of Preventive Medicine, Hyderabad	234 (54·41%)	175 (40·69%)	19 (4·41%)	2 (0·46%)	430
	..	61 (45·52%)	64 (47·76%)	9 (6·71%)	134
Total	535	758	199	16	1508
Percentage	35·5	50·2	13·2	1·1	100

A perusal of Table I would reveal that 35·5% of the vaccine batches had moisture content less than one per cent and 50·2% batches had 1 to 2% moisture

content. In other words majority of the batches (85·7%) had moisture content below 2%. Majority of the Madras and Belgaum vaccines had moisture content less than one per cent. On the other hand, USSR and Patwadnagar vaccines gave rise to percentage moisture between 1 and 2% for over 57% of the batches. Hyderabad vaccines were distributed equally between 1 to 2% and 2 to 3% of moisture content. All this shows that considerable variations exist in respect of moisture content between the sources from which the vaccine batches are obtained.

Two hundred and eighty-six batches whose moisture content was determined were also subjected to two different heat resistance tests (60 batches were kept at 37°C for 28 days and 226 batches subjected to boiling temperature for one hour). Their potency titres were determined on the chorio-allantoic membrane of chick embryo. The thermostability titres were compared with the initial potency titres at —20°C. The data were analysed statistically. The moisture content was classified into three groups, viz. less than one per cent, 1·0 to 1·9% and 2·0% and above. According to the stability of the vaccines as mentioned above the observations were classified at three different levels of moisture content as shown in Tables II and III at 37°C for 28 days as also at boiling temperature for one hour.

Table II. Stability of freezedried smallpox vaccine both indigenous and USSR by moisture content and at 37°C for 28 days

Moisture percentage	Potency titre below $1{\cdot}0\times10^{6}$ PFU/ml on the CAM of chick embryo (Unstable)	Potency titre $1{\cdot}0\times10^{6}$ PFU/ml and above on the CAM of chick embryo (Stable)	Total
<1	1	11	12
1 to 1·9	10	21	31
2·0+	3	14	17
Total	14	46	60

Table III. Stability of freezedried smallpox vaccine both indigenous and USSR by moisture content and at boiling temperature for one hour

Moisture percentage	More than one log fall in potency titre after boiling as compared to initial potency titre at —20°C (Unstable)	Less than one log fall in potency titre after boiling as compared to initial potency titre at —20°C (Stable)	Total
<1	2	28	30
1 to 1·9	9	124	133
2·0+	9	54	63
Total	20	206	226

Statistical analysis was carried out by x^2 test which yielded $x^2(2)=4{\cdot}04$ with $0{\cdot}10<P<0{\cdot}20$ at 37°C for 28 days and $x^2(2)=3{\cdot}19$ with $0{\cdot}20<P<0{\cdot}30$ at boiling temperature for one hour. The results evidently show no significant correlation between variation in moisture content below 4% and heat stability as judged by the above two tests.

Discussion

A few methods have been advocated (Nomura **et al** 1965 and WHO methodology of freezedried smallpox vaccine production) for determining the moisture content of freezedried product but the equipment involved is quite expensive and not easily available. The technique is cumbersome, laborious and time consuming though it may give accurate results. The equipment used in the present investigation is cheap and readily available. The technique is simple and 6-8 batches of the vaccine can be tested for moisture content with fairly good results. The results of moisture content determination test conducted on USSR batches by this method have been found to be more or less in conformity with those mentioned in the protocols supplied along with the USSR vaccine. The results of moisture content in respect of indigenous batches could not be compared with the results of the indigenous vaccine production centres as these centres do not have the facilities to determine the residual moisture content of the freezedried vaccine.

The moisture contents ranging up to 4% do not seem to have any significant effect in lowering the stability of the vaccines. This result conforms to the similar findings by Nomura **et al** (1965).

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