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Rapid Heat Stability (Boiling) Versus Conventional Heat Stability (Standard) Test Of Freeze Dried Smallpox Vaccine

C.L. Sehgal and P. Singha

National Institute of Communicable Diseases Delhi-6

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One hundred and twenty-nine batches of freeze dried smallpox vaccine, indigenous and imported, were subjected to rapid heat stability (boiling) test and conventional heat stability (standard) test to find out whether the rapid test could be an acceptable substitute for the conventional test for practical purposes. The findings of this investigation suggest that the boiling test can be usefully employed as a substitute for conventional test with fairly good, dependable and favourable results from the point of view of economy, time saving and rapidity.

Introduction

Stability of freeze dried smallpox vaccine at high temperature is of great importance especially in tropical and developing countries such as India owing to varied climatic conditions, lack of proper transportation and refrigeration facilities. Thus, a stable freeze dried vaccine is more suitable and of greater significance for National Smallpox Eradication Programme in this country.

World Health Organization (1959, 1966) has recommended conventional heat resistance test on dried smallpox vaccine as the standard test. In this test the dried vaccine is exposed to 37°C for four weeks and then tested for potency on the chorioallantoic membrane of chick embryo. The potency titre must be equal to or greater than 1×10^8PFU/ml. In some countries a more rapid stability test is conducted by heating the dried vaccine at 100°C for one hour. The vaccine passes the test if at least one tenth of the original potency is retained.

The main object of the present study was to find out the potency titre of the dried vaccine batches after subjecting them separately to both the conventional heat stability (CHS) and rapid heat stability (boiling) test and to correlate the results with the original potency at —20°C in order to find out if boiling test could be an acceptable substitute for the CHS test as the former would curtail down the time and the expenditure involved with the latter test. It will be a rapid and practicable test in handling large number of vaccine batches at a time even within the limited laboratory facilities.

Material and Methods

Conventional heat stability (standard) test : Ampoules of freeze dried smallpox vaccine were kept at 37°C for 28 days in a bacteriological incubator and then tested for potency on the chorioallantoic membrane of 12-day old chicken embryos by the technique employed earlier by Sehgal **et al** (1969).

Rapid heat stability (boiling) test : Ampoules of freeze dried smallpox vaccine were kept in the boiling water for one hour and then tested for potency on the chorio-allantoic membrane of 12-day old chicken embryos.

Original potency of vaccine : Ampoules of freeze dried smallpox vaccine after taking out from the deep freeze cabinet at —20 C were tested for original potency on the chorioallantoic membrane of chicken embryos.

Results

In all 129 batches of freeze dried smallpox vaccine, indigenous and imported, were subjected to both the heat stability tests **viz** (*i*) boiling for one hour and (*ii*) kept at 37°C for 28 days. The results of the reading of potency at two different levels of temperature have been shown in Table I.

Table I. Vaccine batches potency at boiling temperature classified according to potency at 37°C

Boiling for one hour	At 37°C for 28 days		Total
	Fall in titre (below 1×10^8 PFU/ml)	No fall in titre (1×10^8 PFU/ml or above)	
Less than one log fall in titre as compared to original titre	5	89	94
More than one log fall in titre as compared to original titre	8	27	35
Total	13	116	129

$\chi^2(1) = 6{\cdot}83$ $\quad 0{\cdot}001 < P < 0{\cdot}01$

In order to examine the relation between the reading at boiling temperature and at 37°C, so far as the potency is concerned, whether a fall of more than one log potency at boiling temperature also leads to the fall of potency at 37°C, the data as shown in Table I were analysed by means of χ^2—test after correction of continuity.

The χ^2 value at one degree of freedom turned out to be 6·83 associated with a probability less than 1 per cent but greater than 0·1 per cent. This shows a significant relation between the readings of potency at two different levels of temperature **viz** boiling and at 37°C. The analysis suggests that if there is a fall of potency

by more than one log at boiling temperature, there is a great likelihood of loss of potency at 37°C.

This point was further analysed statistically by establishing a linear relationship between the difference of potency level at —20°C and at boiling point with the potency level of 37°C, the latter being taken as independent variable. The following analysis of variance (Table II) was worked out considering three levels of potency in terms of log viz 7·50 to 7·99, 8·00 to 8·49 and over 8·50. The result of the analysis of variance establishes this linear relationship between these two variables which supports the result of previous analysis. It is clearly evident that concentration of the points are more closer when the difference of the potency level between —20°C and at boiling point is less than one log and just the opposite viz more divergent when this difference exceeds one log.

Table II. Analysis of variance table for linearity of regression between difference of potency at —20°C and at boiling point with the potency level at 37°C

Source variation	S.S.	d.f.	M.S.	F	P Value
Regression	0·8511	1	0·8511		
Residual	0·0358	1	0·0358	0·256	P>0·05
Between	0·8869	2	0·4435		
Within	17·6014	126	0·1397		
Total	18·4883	128	0·5832		

It has been suggested further by Henderson (1969) that results compiled by W.H.O. show that lots of vaccine which have an initial potency of greater than 3×10^8 PFU/ml on the chorioallantoic membrane and a potency of greater than 3×10^7 PFU/ml after incubation at 100°C for one hour, will consistently meet the standards by the CHS involving incubation of vaccine at 37°C for 28 days. This point was further examined by relating the potency at —20°C classified into two groups viz. (*i*) 3×10^8 PFU/ml or above and (*ii*) less than 3×10^8 PFU/ml with the potency attained at the boiling temperature to see if it is accociated with a fall by one log or more from its original potency at —20°C. One hundred and twenty-nine samples were classified accordingly in Table III by its potency at —20°C cross arranged with the potency attained at boiling point into two groups (*i*) less than 3×10^7 PFU/ml and (*ii*) 3×10^7 PFU/ml or above.

The data were statistically analysed by χ^2—test. The result showed χ^2 was highly significant indicating that if the potency at —20°C was 3×10^8 PFU/ml or more, there is every chance that it will have a potency of 3×10^7 PFU/ml or more after boiling. Similarly, if the potency is less than 3×10^8 PFU/ml at —20°C, there is every likelihood that potency after boiling will be less than 3×10^7 PFU/ml.

Table III. Vaccine batches potency at —20°C cross arranged with the potency at boiling temperature

Potency titre in PFU/ml at —20°C	Potency titre in PFU/ml at boiling temperature		Total
	Less than 3×10^7	3×10^7 or above	
3×10^8 or above	7	63	70
Less than 3×10^8	49	10	59
Total	56	73	129

χ^2 (1) = 66·6 P<·001

The vaccine batches with original potency of 3×10^8 PFU/ml or more at —20°C were also cross classified in Table IV according to their potency result at boiling temperature and at 37°C for 28 days.

Table IV. Vaccine batches with original potency of 3×10^8 PFU/ml or above at —20°C cross classified according to their potency at boiling temperature and 37°C for 28 days

Potency titre in PFU/ml after boiling for one hour	Potency titre in PFU/ml at 37°C for 28 days		Total
	1×10^8 or above	Below 1×10^8	
3×10^7 or above	70	..	70
Below 3×10^7	..	..	..
Total	70	..	70

It has been found invariably that the vaccine batches having potency titre 1×10^8 PFU/ml or above at 37°C yield on boiling temperature a potency titre of 3×10^7 PFU/ml or above. In other words any batch of vaccine having initial potency of 3×10^8 PFU/ml would show a potency of 1×10^8 PFU/ml or above at 37°C will stand the boiling test.

It is with the batches showing initial potency less than 3×10^8 PFU/ml at —20°C that variation in the potency value both on boiling and at 37°C was noted as shown in Table V.

The empirical probability of a batch having potency of 1×10^8 PFU/ml or above at 37°C with initial potency less than 3×10^8 PFU/ml at —20°C passing the boiling test viz. recording a potency of 3×10^7 PFU/ml or above is 45 to 50 per cent. On the other hand batches with the same initial potency viz less than 3×10^8 PFU/ml), showing potency titre less than 1×10^8 PFU/ml at 37°C would invariably fail on boiling test inasmuch as that the probability of such batches recording a potency of less than 3×10^7 PFU/ml is almost 100 per cent.

Table V. Vaccine batches with initial potency less than 3×10^8 PFU/ml at —20°C cross classified according to their potency at boiling temperature and at 37°C

Potency titre in PFU/ml on boiling for one hour	Potency titre in PFU/ml at 37°C for 28 days		Total
	1×10^8 or above	Below 1×10^8	
3×10^7 or above	21	..	21
Below 3×10^7	25	13	38
Total	46	13	59

Discussion

The results of the present investigation indicate that boiling test can substitute the conventional heat stability (standard) test with fairly good and dependable results. Eighty nine (94·7 per cent) out of 94 vaccine batches which showed less than one log fall in titre on boiling as compared to the original potency titre at —20°C did not exhibit any appreciable fall in titre at 37°C for 28 days and were found up to the standard both on rapid and CHS tests. Only 5 (5·3 per cent) out of 94 vaccine batches which showed less than one log fall in titre on boiling as compared to the initial potency titre failed to pass the CHS test. On the other hand, 27 (77·1 per cent) out of 35 vaccine batches which manifested more than one log fall in potency titre on boiling test as compared to initial potency titre at —20°C passed the CHS test, whereas only 8 (22·8 per cent) out of 35 vaccine batches which failed to pass the boiling test also failed on CHS test. In other words, 19 out of 20 batches (95 per cent) which passed the boiling test also passed the CHS test. To accept boiling test will, therefore, not involve a risk of error more than 5 per cent. Against this risk, there is an advantage that accrues from boiling test, in that it is rapid, economical and time saving.

On the other side, 70 (100 per cent) out of 70 vaccine batches which had initial potency titre of 3×10^8 PFU/ml or above at —20°C, showed potency titre of 3×10^7 PFU/ml or more on boiling passed the CHS test showing potency titre 1×10^8 PFU/ml or more. Thus, boiling test for the batches with initial potency of 3×10^8 PFU/ml or more entails no risk of error when it shows a fall in potency titre less than one log on boiling. Twenty-one batches which exhibited initial potency titre less than 3×10^8 PFU/ml at —20°C, showed potency titre of 3×10^7 PFU/ml or more on boiling passed the CHS test showing potency titre of 1×10^8 PFU/ml or above. Twenty-five (65·8 per cent) out of 38 vaccine batches which had initial potency titre less than 3×10^8 PFU/ml, also showed potency titre less than 3×10^7 PFU/ml on boiling but passed the CHS test showing potency titre of 1×10^8 PFU/ml or more. But 13 (34·2 per cent) out of 38 vaccine batches which exhibited initial potency titre less than 3×10^8 PFU/ml at —20°C also showed potency titre less than 3×10^7 PFU/ml on boiling and potency titre less than 1×10^8 PFU/ml on CHS test and thus did not conform to the standard laid down by the W.H.O. (1959, 1966).

Although 66 per cent of the batches which show on boiling potency titre below 3×10^7 PFU/ml, pass the CHS test but 34 per cent batches fail to do so. As the amount of element of risk is very high, it is advisable to resort to CHS test on the vaccine batches which on boiling show potency titre less than 3×10^7 PFU/ml.

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