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POTENCY AND STABILITY CHARACTERISTICS OF SMALLPOX VACCINE USED IN THE  
SMALLPOX ERADICATION PROGRAMME IN WESTERN AND WEST-CENTRAL AFRICA

by

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The virtual eradication of smallpox in West and West-Central Africa is evidence that the objective of more than 200 years of man's effort to control this disease can be achieved. The success of this five-year programme is due to meticulous surveillance, efficient vaccination and use of vaccine that withstood the severity of a tropical environment. The purpose of this report is to document the potency and stability characteristics of the vaccine with observations relative to laboratory assays and potency in man.

Smallpox vaccine

Wyeth's smallpox vaccine, (Dryvax<sup>®</sup>), used in the African programme is a lyophilized calf lymph product made from the 22nd to 28th heifer passage of the New York City Department of Health Strain of vaccinia virus and contains peptone as a stabilizing agent. Three-fourths of the 180 million doses prepared by Wyeth were for administration by jet gun; the remainder for use with Wyeth's bifurcated needle. All lots of vaccine complied with the United States Public Health Service (USPHS) and WHO requirements.

Since April 1971, the USPHS requirements have included the counting of pocks formed on the chorioallantoic membrane of embryonated chicken eggs (CAM assay) as an alternative to the rabbit scarification (RS) assay procedure. The USPHS potency requirement for the product specifies that the CAM assay titre be at least equivalent to the Reference Vaccine in a simultaneous assay. WHO recommends a titre in excess of  $8.0 \log_{10}$  Pock Forming Units (PFU's) per ml.

The United States Reference Smallpox Vaccine Lot 2 (Reference Vaccine) made by Wyeth is a lyophilized calf lymph preparation in boro-silicate ampoules sealed under vacuum and stored by the regulatory agency at  $-70^{\circ}\text{C}$  since its manufacture in 1958.

CAM assay

Vaccine produced by Wyeth for use in Africa was tested for potency by the RS and CAM assays. The latter was standardized to the use of 12-day old embryonated eggs inoculated with 0.1 ml of  $10^{-6.0}$  and  $10^{-6.2}$  dilutions of both production and Reference Vaccines using eight to 10 eggs per dilution. Since the target titre for the vaccine is 8.0 to  $8.5 \log_{10}$  PFU's per ml, the procedure resulted in the formation of 10 to 30 pocks per membrane.

The mean titre of the Reference Vaccine based on 399 CAM assays over 5-1/2 years was  $8.3 \log_{10}$  PFU's per ml with a standard deviation of 0.13. An assay was considered valid if the titre of the Reference Vaccine was not less than 8.0 and not more than  $8.6 \log_{10}$  PFU's per ml. Production lots of vaccine were considered equivalent to the Reference if the titre was not less than  $8.0 \log_{10}$  PFU's per ml.

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Vaccine rehydrated for administration by jet gun is a 33-fold ( $1.5 \log_{10}$ ) dilution of vaccine intended for administration with a bifurcated needle. The titres of 77 lots of vaccine for jet gun use ranged from 6.5 to 7.1  $\log_{10}$  PFU's per ml; 82% of which were greater than 6.7  $\log_{10}$  PFU's per ml. The titres of 94 lots of vaccine for use with the bifurcated needle ranged from 8.0 to 8.6  $\log_{10}$  PFU's per ml, 82% of which were greater than 8.2  $\log_{10}$  PFU's per ml.

During the initial phase of utilizing the CAM assay procedure, we observed that the source of embryonated eggs, the age of embryo and the volume of inoculum affected the assay titre.

The CAM assay titres of the Reference Vaccine obtained with 12-day old embryonated eggs from different flocks using an inoculum of 0.1 ml are shown in Table 1. The titres obtained with eggs from three flocks from Supplier A were 0.6 to 0.8  $\log_{10}$  lower than the titres obtained with eggs from another supplier or with eggs from Wyeth's RIF-free flock. Studies were not undertaken to determine whether the reduced sensitivity of the eggs was related to genetic or environmental factors such as composition of feed and sanitizing practices.

In ascertaining the influence of age of the embryo on the infectivity titre of the Reference Vaccine, eggs from a single flock were used. Five assays were performed at different times with 10, 11, 12, 13 and 14-day old eggs. The data in Part A of Table 2 indicate that 1.0 and 0.5  $\log_{10}$  lower titres were obtained with 10 and 11 day old eggs respectively as compared to the titres obtained with 12-day old embryos. The titres obtained with 13 and 14-day old embryos were about 0.2  $\log_{10}$  higher. Eggs used in this study were set for incubation the day they were laid to avoid a possible variation related to the 21-day interval in which fertile eggs may be stored.

Part B of Table 2 summarizes the data obtained with routinely supplied 11, 12 and 13-day old embryos. The standard deviation of the mean titre of the Reference Vaccine obtained with 11-day old embryos was slightly more than twice the value obtained with 12 and 13-day old embryos. This suggests that 11-day old embryos are not as uniform in sensitivity as older aged embryos. The difference in titres obtained with 11 and 13-day old embryos is not statistically significant because of the greater standard deviation obtained with 11-day old embryos.

In a series of assays of the Reference and production lots of vaccine using inoculum volumes of 0.1 and 0.2 ml, the infectivity titres obtained with the smaller inoculum were consistently 0.1 to 0.3  $\log_{10}$  higher. The report of SLONIM et al. (2) that higher titre values were obtained with a 0.025 ml inoculum than with 0.1, 0.2 or 0.4 ml, stimulated us to expand our studies.

The data in Table 3, representing four independent assays in which inocula were carefully dispensed with 1 ml tuberculin syringes, confirms the observations of SLONIM et al. In another study, serial two-fold dilutions of vaccine were assayed using inocula of 0.025, 0.1 and 0.2 ml. Appropriate dilutions of individual serial dilutions of vaccine were inoculated to obtain 10 to 30 pocks per membrane. For each sample assayed, the titre obtained with the 0.025 ml inoculum was higher than that obtained with 0.1 ml or 0.2 ml. Parallel slopes were obtained for each of the inocula as shown in Figure 1. Similar observations have been made in assaying lyophilized vaccine stored at 35°C for 30 days.

The combined effect of the volume of inoculum and age of embryonated eggs on CAM assay titre of the Reference Vaccine is shown in Table 4. The highest average titre was obtained in 13-day old embryos using 0.1 ml inoculum whereas the lowest was obtained in 11-day old embryos with an inoculum of 0.2 ml; a difference of 0.9  $\log_{10}$ . Again, the standard deviation of the mean titre obtained with 11-day old embryos was twice that obtained with the older embryos.

The data presented concerning the effect of the source of eggs, the age of embryos and volume of inoculum may, as individual factors, be of slight significance in establishing potency of a vaccine, but when combined, may result in an underestimated value. The use of a reference vaccine for each titration and the establishment of criteria of test validity based on a standardized procedure permit a better estimate of the potency and stability characteristics of smallpox vaccine.

#### Clinical studies

Laboratory assay data of biological products are significant only if the observations have been related to potency in man. For smallpox vaccine, this would mean protection against disease, formation of neutralizing antibody and the development of a major reaction at the site of vaccination.

We recognized a need to relate the potency of the United States Reference Smallpox Vaccine Lot 2 to performance in man and elected to compare the skin response of young adult revaccines to the rabbit scarification test ratio and the CAM assay titre of undiluted and diluted preparations of the Reference Vaccine.

ESPMARK (1) established that less infectious virus is required to obtain a major reaction in primary vaccinees than in revaccinees and that the concentration of virus required to produce a major reaction in revaccinees varied inversely with the interval since their last vaccination. Therefore, administration of vaccine to young adult revaccinees would be a stringent test for potency.

Our study population was from a correctional institution for males 15 to 22 years of age. A smallpox vaccination is required for all incoming citizens including those entering the institution for a second or third time. Some of the older inmates had been in military service and received a smallpox vaccination at the time of induction. Virtually all had received a primary vaccination between one and six years of age. For approximately one-half of the population, the estimated time interval since their previous vaccination ranged from 10 to 20 years. To control this variable, each individual was administered two vaccinations by an experienced vaccinator (MZB), a test preparation on one arm and undiluted Reference Vaccine on the other arm as the control. The vaccine was administered with Wyeth's bifurcated needle using 15 to 20 tangential pressures.

Two coded samples of rehydrated Reference Vaccine were prepared on the day of immunization; the undiluted Reference Vaccine and the other, either a dilution of the Reference Vaccine or an undiluted preparation of the same. The last two preparations are identified as test vaccine whereas the first one is identified as the control vaccine. The vaccinator was not aware of the identity of the samples at time of vaccination or examination of arms one week later. The reactions were recorded as either major or equivocal as defined by the WHO Expert Committee on Smallpox (3) and tabulated as the number of individuals that developed major reactions with each of the coded vaccine preparations. The results as shown in Table 5 were also converted to a ratio of the percentage of individuals that developed a major reaction on the arm that received the test vaccine (diluted or undiluted Reference Vaccine) to the percentage of individuals that had major reactions on the arm that received the control, undiluted Reference Vaccine.

The susceptibility of the 18 groups vaccinated over a period of 15 months were similar as indicated by the percentage of individuals that developed major reactions with the control vaccine (undiluted Reference Vaccine). Sixty-three per cent. of the total population developed a major reaction following administration of the control vaccine and with the exception of the group of 20 individuals that received the 1/100 dilution of test vaccine, the response of the other groups did not vary more than 8% from that of the total.

Of the 87 individuals that received undiluted Reference Vaccine on both arms, there was 94% agreement in the formation of major reactions at the two sites; 53 individuals developed major reactions on one arm and 50 had a similar response on the other arm. In the four individual trials comprising the undiluted group, there was 82%, 96% and 100% (two instances) agreement. The individuals that received either 1/2.5 or 1/3.5 dilution of vaccine on one arm and control vaccine on the other, experienced the same frequency of major reactions on both arms. Although the average ratio of test vaccine to control vaccine responses for the groups administered the 1/5 and 1/10 dilutions of vaccine were within the range observed with the group that received the control preparation on both arms, there was one trial in each group that had ratios of 0.64 and 0.78 respectively. The marked difference in the responses obtained with the 1/100 dilution of Reference Vaccine indicated that there was no need to carry out further observations.

Aliquots of the test and control preparations were tested for potency by the rabbit scarification and CAM assays as coded samples. These potency test data and responses obtained in revaccinees are presented in Table 6. The data indicate that vaccine with a titre of  $7.7 \log_{10}$  PFU's per ml or a rabbit scarification test ratio of 0.5 will produce the same frequency of major reactions in young adult revaccinees as the United States Reference Smallpox Vaccine lot that has a CAM assay titre of  $8.3 \log_{10}$  PFU's per ml, when administered by multiple pressure technic with Wyeth's bifurcated needle.

#### Stability studies

One of our early studies concerning the stability characteristics of lyophilized vaccine consisted of an evaluation of the two types of vaccine, i.e. for administration by jet gun or by bifurcated needle. Samples of 11 lots of vaccine stored at 100°C, 70°C, 50°C, 37°C, 25°C, 5°C and -20°C were assayed at various time intervals in primary cercopithecus monkey kidney (CMK) cell cultures.

On the basis of 22 assays of the Reference Vaccine, the 95% confidence limits of a smallpox vaccine assay titre by the CMK cell culture system are  $\pm 0.4 \log_{10}$  TCID<sub>50</sub> per ml. Since the observations obtained for each of the 11 lots in this study were similar, the combined results are presented in Figures 2 and 3. Significant reduction in the titre of vaccine ( $0.5 \log_{10}$  TCID<sub>50</sub> per ml) occurred following one hour storage at 100°C, one day at 70°C, two weeks at 50°C, six months at 37°C and 21 months at 25°C.

In our laboratory, the infectivity titre of smallpox vaccine obtained by the CMK cell culture system expressed as  $\log_{10}$  TCID<sub>50</sub>'s per ml is comparable to the titre values obtained by the CAM assay. The correlation of the two assay systems is presented in Figure 4. However, this may not be a universal finding since comparable agreement was not obtained in assaying the International Reference Standard by the two systems. The observed differences in titres as shown in Table 7 may be related to the strain of virus used to prepare the different references. The United States and Wyeth References were made with the NYC Board of Health strain whereas the International Reference was prepared with the Lister strain.

A second study was undertaken to establish the stability characteristics of Wyeth's lyophilized vaccine with the CAM assay procedure. The data presented in Figure 5 describes the average change in titre for six lots of vaccine stored at 37°C, 25°C and 5°C as compared to the titre of vaccine stored at -20°C assayed at the same time intervals. The titres of vaccine stored at 25°C showed a gradual decrease in titre of 0.3-0.4  $\log_{10}$  PFU's per ml. Following three months of storage at 37°C the potency was reduced 0.4  $\log_{10}$  and after six months the titre was 0.5  $\log_{10}$  lower than the control vaccines. These findings are quite similar to those obtained with the CMK cell system assays and confirm the interchangeability of the two assay systems.

Long-term stability data for vaccine stored at 2-8°C were obtained by assaying samples of 27 production lots stored for 1-1/2 to 8-1/2 years. The seven oldest lots were, at the time of manufacture, tested for potency by the rabbit scarification test. These lots were first assayed in embryonated eggs in 1968 and again three years later. The CAM assay titre of the other 20 lots at time of manufacture ranged from 8.0 to 8.6 log<sub>10</sub> PFU's per ml. The titres of the seven oldest lots when assayed in 1968 ranged from 8.0 to 8.3 log<sub>10</sub> PFU's per ml. The titre values for the 27 lots of vaccine in relationship to the time of storage presented in Figure 6 indicate that lyophilized vaccine may be stored as long as 8-1/2 years at 2-8°C without significant loss of potency.

A study was undertaken to define the stability characteristics of vaccine rehydrated for use with the bifurcated needle. The study was designed to simulate use of the product in a physician's office or immunization clinic where it may be exposed to ambient temperatures for various time intervals until the material is depleted. Samples of six lots of rehydrated vaccine were stored at:

- A. 2-8°C only;
- B. 2-8°C with 2-1 hour intervals at 25°C per week for the initial two months and for one hour at 25°C per week during the last two months. Total time at 25°C was 24 hours in 16 weeks;
- C. 2-8°C with 20 minutes at 25°C five days per week. Total time at 25°C was 26 hours in 16 weeks, and
- D. -10 to -20°C only.

All vaccines were assayed at four-week intervals. Since the titres of the six lots at time of manufacture were almost identical (8.3, 8.4, 8.3, 8.5, 8.4 and 8.5 log<sub>10</sub> PFU's/ml) the data have been consolidated and are presented as average values in Table 8. Vaccines stored under the four experimental conditions maintained potency titres equivalent to the Reference Vaccine for 16 weeks. Some of the vaccines were administered as coded samples to young adult revaccinees with a bifurcated needle. A coded sample of Reference Vaccine was administered to the other arm as previously described. As shown in Table 9, the ratio of the percentage of major reactions that developed following administration of test vaccine (B) to the percentage obtained with Reference Vaccine (A) ranged from 0.87 to 1.09 during the 17 weeks of observation. At 15, 16 and 17-week intervals, the ratios were 1.0, 1.0 and 0.94 respectively confirming the laboratory assays that Wyeth's smallpox vaccine maintains adequate potency following rehydration and storage at 2-8°C for four months.

Table 10 summarizes the results of three studies establishing the stability characteristics of vaccine rehydrated for administration by jet gun. The study evaluated the use of diluent without preservative which was supplied for use in Africa, and diluent containing 0.25% phenol. Vaccine rehydrated with diluent without phenol did not lose titre after storage at 37°C for 32 hours. Vaccine rehydrated with diluent containing phenol showed a 0.3 log<sub>10</sub> reduction in titre during the same time interval.

#### Comment and summary

We have presented evidence indicating that in assaying the potency of smallpox vaccine by the CAM procedure, lower titre value may be obtained depending upon the source of embryonated eggs, the age of the embryos, and volume of the inoculum. For this reason, it is desirable to standardize the assay with use of 12 to 13-day old embryos, an inoculum of 0.1 or 0.05 ml and simultaneous titration of a reference vaccine to evaluate the sensitivity of the assay.

The WHO and USPHS Smallpox Vaccine requirements have at least a two to four-fold excess potency factor. This is indicated by our observations that vaccine with a titre of  $7.7 \log_{10}$  PFU's per ml will produce the same frequency of major reactions in young adult revaccinees as vaccine with a titre of  $8.3 \log_{10}$  PFU's per ml. The excess potency factor is surely greater in the case of primary vaccinees.

The successful smallpox eradication programme in a segment of Africa corroborates the results of stability studies indicating that lyophilized and rehydrated vaccines would maintain adequate potency when stored and used as directed.

#### REFERENCES

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TABLE 1  
CAM ASSAY TITERS OF U.S. REFERENCE SMALLPOX VACCINE LOT 2  
USING EMBRYONATED EGGS FROM DIFFERENT FLOCKS

<u>SOURCE OF EGGS</u>	<u>NO. OF ASSAYS</u>	<u>MEAN TITER</u> <u>LOG<sub>10</sub> PFU's/ml.</u>
SUPPLIER A		
FLOCK We	7	7.7
FLOCK Br	8	7.5
FLOCK Wa	1	7.7
SUPPLIER B	2	8.4
WYETH'S RIF- FREE FLOCK	3	8.3

TABLE 2  
INFLUENCE OF AGE OF EMBRYONATED EGGS ON THE CAM ASSAY TITER OF  
U.S. REFERENCE SMALLPOX VACCINE LOT 2

A. EMBRYONATED EGGS SET ON DAY LAYED

<u>AGE OF EMBRYO</u>	<u>DIFFERENCE IN TITER* OBTAINED WITH 12 DAY OLD EMBRYOS</u>					<u>AVERAGE</u>
	<u>ASSAY 1</u>	<u>ASSAY 2</u>	<u>ASSAY 3</u>	<u>ASSAY 4</u>	<u>ASSAY 5</u>	
10 DAYS	-1.0		-1.0		-1.1	-1.0
11 DAYS	-0.4	-0.3	-0.6	-0.6	-0.5	-0.5
12 DAYS	8.3*	8.3	8.2	8.3	8.2	
13 DAYS	+0.2	+0.3	+0.2	+0.3	+0.2	+0.2
14 DAYS	+0.3	+0.2	+0.2	+0.3	+0.2	+0.2

\*LOG<sub>10</sub> PFU's per ml.

B. ROUTINELY SUPPLIED EMBRYONATED EGGS

<u>AGE OF EMBRYO</u>	<u>NO. OF ASSAYS</u>	<u>TITER - LOG<sub>10</sub> PFU's/ MI.</u>			
		<u>MEAN</u>	<u>RANGE</u>	<u>STAND. DEVIATION</u>	<u>95% CONF. LIMITS</u>
11 DAYS	12	8.0	7.6 - 8.4	0.28	± 0.5
12 DAYS	125	8.4	8.1 - 8.6	0.11	± 0.2
13 DAYS	17	8.6	8.4 - 8.8	0.11	± 0.2

TABLE 3  
EFFECT OF VOLUME OF INOCULUM ON THE CAM ASSAY OF U.S.  
REFERENCE SMALLPOX VACCINE LOT 2 \*

DILUTION OF VACCINE INOCULATED	INOCULUM VOLUME - Mls.			
	<u>0.2</u>	<u>0.1</u>	<u>0.05</u>	<u>0.025</u>
$10^{-5.8}$	** 7.9 (26) a	8.1 (20)	8.2 (13)	8.3 (8)
$10^{-6.0}$	7.9 (17)	8.2 (16)	8.3 (11)	8.5 (9)
$10^{-6.2}$	8.1 (15)	8.2 (11)	8.4 (7)	8.8 (10)

\* COMBINED DATA OF 4 ASSAYS

\*\* INFECTIVITY TITER  $\text{LOG}_{10}$  PFU's PER ml.

a AVERAGE NUMBER OF POCKS PER MEMBRANE

TABLE 4  
COMBINED EFFECT OF VOLUME OF INOCULUM AND AGE OF  
EMBRYONATED EGGS ON THE CAM ASSAY TITER OF U.S. REFERENCE SMALLPOX VACCINE LOT 2

VOLUME OF INOCULUM		AGE OF EMBRYONATED EGGS		
		<u>11 DAYS</u>	<u>12 DAYS</u>	<u>13 DAYS</u>
<u>0.1 ml.</u>	AVERAGE TITER $\text{LOG}_{10}$ PFU's/ml.	8.2	8.4	8.7
	NO. OF ASSAYS -	4	93	6
	STANDARD DEVIATION	0.23	0.11	0.07
<u>0.2 ml.</u>	AVERAGE TITER $\text{LOG}_{10}$ PFU's/ml.	7.8	8.2	8.5
	NO. OF ASSAYS -	14	15	3
	STANDARD DEVIATION	0.21	0.11	0.07

TABLE 5

RESPONSES OF YOUNG ADULT REVACCINEES TO VACCINATION WITH UNDILUTED & DILUTED PREPARATIONS OF U.S. REFERENCE SMALLPOX VACCINE LOT 2 ADMINISTERED WITH WYETH'S BIFURCATED NEEDLE

Dilution of U.S. Ref. Smallpox Vac. Lot 2 Test Vaccine	No. of Trials	No. of Vaccinees	No. Major Reactions on Arms That Rec'd		Ratio B/A	
			Control ** Vaccine A	Test Vaccine B	Average	Range in Trials
UNDILUTED	4	87	53 (61%)*	50	0.94	0.82 - 1.00
1/2.5	2	27	18 (67%)	17	0.96	0.93 - 1.00
1/3.5	2	47	26 (55%)	25	0.96	0.92 - 1.00
1/5.0	6	153	99 (65%)	84	0.85	0.64 - 1.11
1/10	3	68	43 (65%)	37	0.85	0.78 - 0.93
1/100	1	20	15 (75%)	3	0.2	
TOTAL	18	402	254 (63%)			

- \* Percent of individual developing major reactions on arm that received control vaccine.
- \*\* Undiluted U.S. Reference Smallpox Vaccine Lot 2

TABLE 6

CORRELATION OF POTENCY ASSAY VALUES OF UNDILUTED & DILUTED U.S. REFERENCE SMALLPOX VACCINE LOT 2 WITH SKIN RESPONSES IN YOUNG ADULT REVACCINEES ADMINISTERED VACCINE WITH WYETH'S BIFURCATED NEEDLE

Dilution of U.S. Ref. Smallpox Vac. Lot 2	No. of Trials	CAM Assay Log <sub>10</sub> PFU's/ML.		Rabbit Scar'tn Test Ratio to Reference*		Development of Major Reactions Ratio $\frac{\text{Test Vaccine B}}{\text{Cont. Vaccine A}}$
		Avg.	Range	Avg.	Range	
<b>CONTROL VAC. A</b>						
Undiluted	18	8.3	8.1 - 8.6	0.93	0.82 - 1.05	
<b>TEST VACCINE B</b>						
Undiluted	4	8.3	8.2 - 8.4	0.94	0.86 - 1.03	0.94
1/2.5	2	8.0	8.0 - 8.1	0.54	0.61 - 0.46	0.96
1/3.5	2	7.7	7.7 - 7.8	0.52	0.58 - 0.46	0.96
1/5.0	6	7.6	7.2 - 7.7	0.38	0.24 - 0.48	0.85
1/10	3	7.4	7.4, 7.4, 7.4	0.24	0.21 - 0.27	0.75
1/100	1	6.2		0.03		0.2

- \* U.S. Reference Smallpox Vaccine Lot 2 - rehydrated at time of assay

TABLE 7  
CAM AND CMK TISSUE CELL CULTURE ASSAY OF THE INTERNATIONAL AND  
U.S. REFERENCE SMALLPOX VACCINES

<u>REFERENCE</u>	<u>CAM ASSAY</u> <u>LOG<sub>10</sub> PFU's/ml.</u>	<u>CMK ASSAY</u> <u>LOG<sub>10</sub> TCID<sub>50</sub>/ml.</u>	<u>TITER RATIO</u> <u>CMK/CAM</u>
INTERNATIONAL REFERENCE	8.3	7.4	- 0.9
U.S. REFERENCE Smallpox Vaccine Lot 2	8.4	8.8	+ 0.4
WYETH REFERENCE Vaccine	7.9	8.2	+ 0.3

TABLE 8  
REHYDRATED DRYVAX® STABILITY STUDY

<u>STORAGE</u> <u>TIME</u> <u>WEEKS</u>	<u>CAM Assay Titers - Log<sub>10</sub> PFU's per MI.</u>				<u>U. S. REFERENCE</u> <u>SMALLPOX VACCINE</u> <u>LOT 2</u>
	<u>Storage Conditions *</u>				
	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	
0	8.4				
4	8.4	8.3	8.4	8.5	8.2
8	8.4	8.2	8.3	8.4	8.3
12	8.2	8.2	8.2	8.4	8.3
16	8.2	8.1	8.2	8.4	8.3
					8.2

\* Storage Conditions Dryvax® - Lyophilized smallpox vaccine

- A 2-8°C only
- B 2-8°C with 2 - 1 hour intervals at 25°C per week
- C 2-8°C with 20 - minute intervals at 25°C 5 days per week
- D At - 10 to -20°C only

TABLE 9

SKIN RESPONSES IN YOUNG ADULT REVACCINEES ADMINISTERED\*  
REHYDRATED DRYVAX® STORED AT 2-8°C WITH WYETH'S BIFURCATED NEEDLE

Storage Time Weeks	CAM Assay Titer Log <sub>10</sub> PFU's/ml.	No. of Vaccinees	Percent Major Reactions				Ratio B/A
			Ref. Vaccine A**		Test Vaccine B		
			No.	(%)	No.	(%)	
0	8.4	86	62	(72)	59	(69)	0.96
4	8.1	60	37	(62)	34	(57)	0.92
8	8.1	33	22	(67)	24	(73)	1.09
10	8.1	29	24	(83)	22	(76)	0.92
12	8.2	49	39	(80)	34	(69)	0.87
13	7.9	73	46	(63)	41	(56)	0.89
15	8.0	21	13	(62)	13	(62)	1.00
16	8.0	36	23	(64)	23	(64)	1.00
17	8.2	30	20	(67)	19	(63)	0.94

\* Storage Condition B see text

\*\* U.S. Reference Smallpox Vaccine Lot 2 - 8.4 Log<sub>10</sub> PFU's per ml  
Dryvax® - Lyophilized smallpox vaccine

TABLE 10

STABILITY CHARACTERISTICS OF SMALLPOX VACCINE REHYDRATED FOR  
ADMINISTRATION WITH A JET GUN

STORAGE		CHANGE IN TITER (LOG <sub>10</sub> TCID <sub>50</sub> /ml.) <sup>a</sup> FROM ZERO TIME	
TEMPERATURE	TIME	DILUENT WITHOUT PHENOL	DILUENT WITH 0.25% PHENOL
5°C	8 HOURS	0.0	- 0.1
	24 HOURS	-0.1	- 0.1
	32 HOURS	0.0	- 0.1
25°C	8 HOURS	0.0	- 0.1
	24 HOURS	0.0	- 0.2
	32 HOURS	-0.1	- 0.2
37°C	8 HOURS	0.0	- 0.2
	24 HOURS	0.0	- 0.3
	32 HOURS	0.0	- 0.3
18 hours at 37°C plus 6 hours at 25°C		0.0	- 0.3

<sup>a</sup> Combined Data of 3 Studies using cercopithecus monkey kidney tissue cell cultures

FIGURE 1  
CAM ASSAY TITERS USING 0.2, 0.1, and 0.025 ML.  
AS THE VOLUME OF INOCULUM

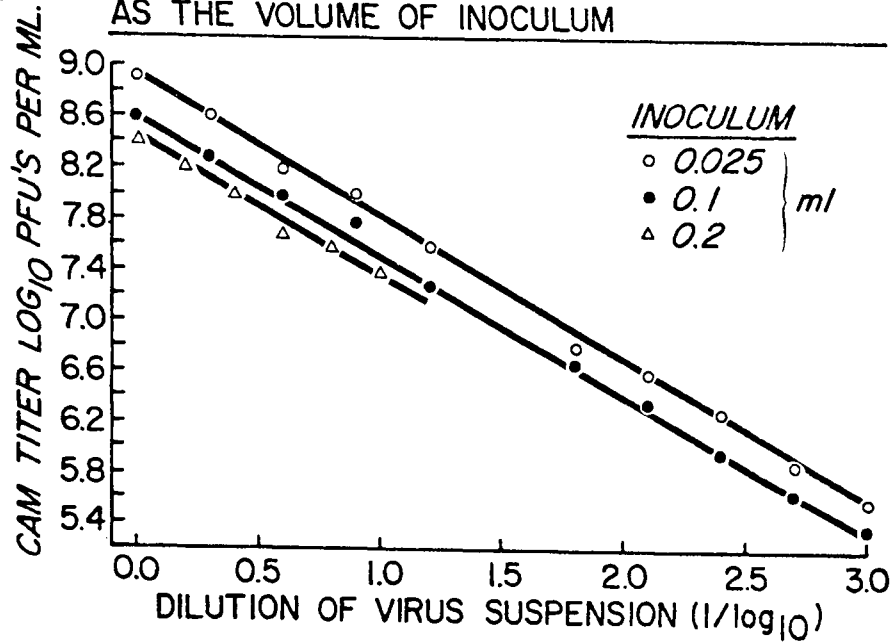
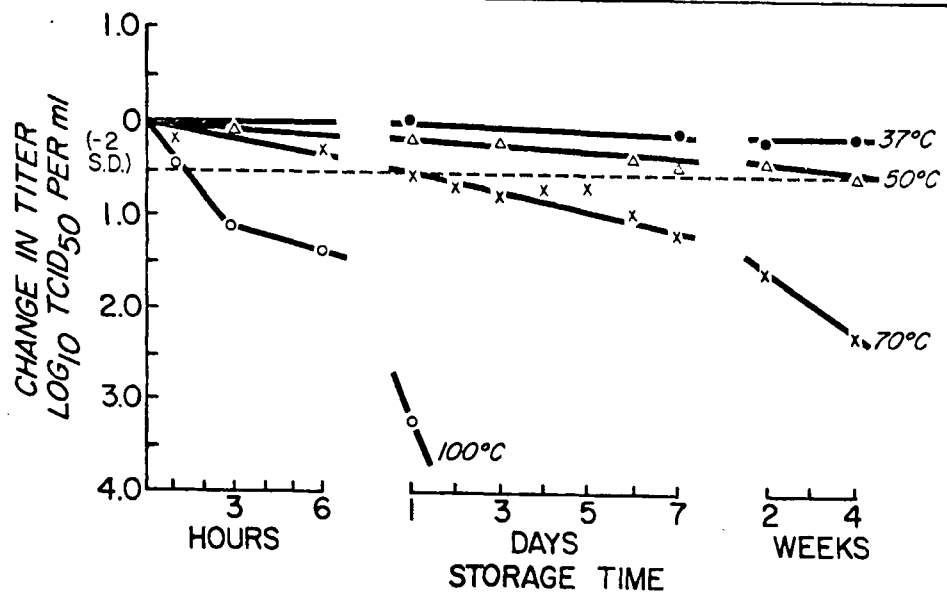


FIGURE 2  
DRYVAX® STABILITY STUDIES - LYOPHILIZED VACCINE STORED  
AT 37°, 50°, 70°, & 100°C. - CMK TISSUE CELL ASSAY



DRYVAX® - Lyophilized smallpox vaccine

FIGURE 3

DRYVAX® STABILITY STUDIES-LYOPHILIZED VACCINES STORED AT 5°, 25°, 37°, 50°, & 70°C. - CMK TISSUE CELL ASSAY

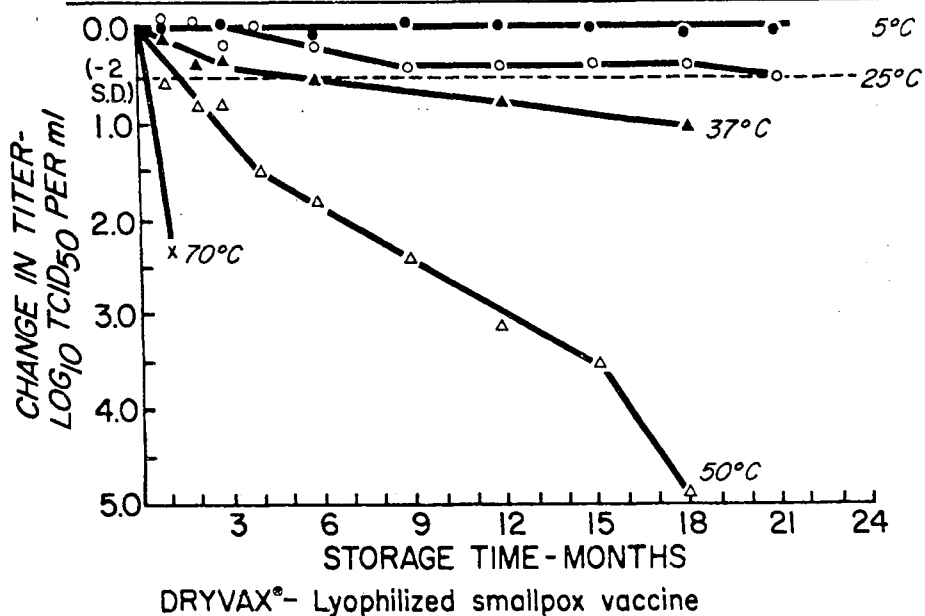


FIGURE 4

COMPARISON OF CMK TISSUE CELL & CAM ASSAYS OF SERIAL TWO-FOLD DILUTIONS OF SMALLPOX VACCINE

