## 1. Calibration of laboratory reference standard

The lyophilized rabbit brain rabies vaccine applied for laboratory reference standard, were triplicate calibrated the potency by comparison with the third Internation Reference Preparation of rabies vaccine. The potency of vaccines are shown in Table II. Average potency were calcululated as geometric mean of $0.6 \mathrm{IU} / \mathrm{ml}$. From the result also shows that these lyophilized products had regularly dispersed suitable for being the laboratory reference standard.
2. Potency test of vaccine

### 2.1 Semple vaccine

The potency of tested Semple vaccines were shown in Table III ( $\mathrm{A}-\mathrm{E}$ ).

2.2 Suckling mouse brain vaccines (SMBV)

The potency of SMBV in this test were shown in Table IV (A and B) An accelerated, degradation test were the method to test the stability of vaccine in suspension media, the results were shown in Table V (A and B)

### 2.3 Lyophilize suckling mouse brain vaccines

The lyophilized vaccines were tested for potency and stability, the results were shown in Table IX.

In Table III, IV illustrates the effect of inactivation methods on vaccine potency. Inactivated by heat, vaccine preparation resulted the antigenic potency higher than inactivated by phenol, but lower than BPL.
'In comparison of the effect of various suspension media on vaccine potency and stability, no significant differences were observed as shown in Table III to Table VIII.

The contrast of potency in lyophilized suckling mouse brain vaccines that shown in Table IX indicates the effect of stabilizer, though lactose saline dealt' with higher potency as sucrose saline and dextransaline. But vaccine was more stable in sucrose saline.


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Table II : Calibration of the Potency of Laboratory Reference Standard


Table III A : Comparison of the potency of semple vaccine $10 \%$ suspension using NSS and PES , as suspension media and inactivated by various methods.


Table III B : Comparison of the potency of semple vaccine 10 suspension using NSS and PBS as suspension media and inactivated by various methods.

| Vaccine | Dilution | Surv ived | Died | Cummulative |  | $\left\{\begin{array}{c} z \\ \text { Mortality } \\ . \end{array}\right.$ | $\begin{gathered} 50 \mathrm{z} \\ \text { End } \\ \text { point } \end{gathered}$ | Potency |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | Surv ived | Died |  |  |  |
| Laboratory <br> Reference <br> Standard | 1:5 | 0 |  | 0 <br> 5 <br> 17 <br> 32 | 32 | 0 | $10^{-1.74}$ |  |
|  | 1:25 |  |  |  |  | 24 |  |  |
|  | 1:125 | 12 | 4 |  |  | 77 |  |  |
|  | 1:625 | 15 | 1 |  |  | 97 |  |  |
| B-PS | 1:5 | 1514 |  | 1 <br> 6 <br> 20 | 28 <br> 13 <br> 2 |  |  |  |
|  | $\begin{aligned} & 1: 25 \\ & 1: 125 \end{aligned}$ |  |  |  |  | 32 | $10^{-1.61}$ | $0.44 \mathrm{IV} / \mathrm{mL}$ |
|  |  |  |  |  |  | 91 |  |  |
| P-PS | $\begin{aligned} & 1: 5 \\ & 1: 25 \\ & 1: 125 \end{aligned}$ |  |  | 2 <br> 10 <br> 25 |  | 8 |  |  |
|  |  |  |  |  |  | 53 | $10^{-1.35}$ | $0.24 \mathrm{IV} / \mathrm{mL}$ |
|  |  |  |  |  |  | 96 |  |  |
| H-PS | $\begin{aligned} & 1: 5 \\ & 1: 25 \\ & 1: 125 \end{aligned}$ | 1 <br> 7 <br> 12 | $15$ |  |  |  |  |  |
|  |  |  |  |  | 13 | 38 | $10^{-1.58}$ | $0.42 \mathrm{IU} / \mathrm{mL}$ |
|  |  |  |  | 20 | 12 | 83 |  |  |
|  | $1: 5$$1: 25$$1: 125$ | 0 <br> 14 | 16 <br> 11 <br> 2 | - 0 | 29 | 0 |  |  |
|  |  |  |  | $\square 5$ |  | 28 | $10^{-1.65}$ | $0.49 \mathrm{IU} / \mathrm{mL}$. |
|  |  |  |  |  |  | 91 |  |  |
| P-NS | $\begin{aligned} & 1: 5 \\ & 1: 25 \\ & 1: 125 \end{aligned}$ |  | 13 1 <br> 9 10 |  | 23 <br> 10 | 12 |  | $0.27 \mathrm{IU} / \mathrm{mL}$ |
|  |  |  |  |  | 50 | $10^{-1.40}$ |  |  |
|  |  |  | 1 | 25 |  | 1 | 96 |  |  |
| H-NS | $\begin{aligned} & 1: 5 \\ & 1: 25 \\ & 1: 125 \end{aligned}$ |  | 16 | 0 | 28 | 4083 | $10^{-1.56}$ | $0.40 \mathrm{IU} / \mathrm{mL}$ |
|  |  |  | 8 | 8 | 12 |  |  |  |
|  |  |  | 4 | 20 |  |  |  |  |

Table III C : Compaitson of the potency of semple vaccine $10 \%$ suspension using NSS and PBS
as suspension media and inactivated by various methods.


Remark : Virus Titre $=10^{-6.45}$
B-NS $=$ Betapropiolactone inactivated in normal saline (NSS)
B-PS. = Betapropiolactone inactivated in phosphate buffer saline (PBS)
H-NS = Heat inactivated in NSS
H-PS $=$ Heat inactivated in PBS
P-NS = Phenol inactivated in NSS
P-PS $=$ Phenol inactivated in PBS

Table III D : Comparison of the potency of semple vaccine 10 suspension using NSS and PBS as suspension media and inactivated by vaxious methods.


Table III E : Comparison of the potency of semple vaccine 10 suspension using NSS
and PBS as suspension media and inactivated by various methods


Remark : Virus Titre $=10^{-6,5}$
$B-N S=$ Betapropiolactone inactivated in normal saline (NSS)
B-PS = Betapropiolactone inactivated in phosphate buffer saline (PBS)
H-NS $=$ Heat inactivated in NSS
H-PS $=$ Heat inactivated in PBS
P-NS $=$ Phenol inactivated in NSS
$\mathrm{P}-\mathrm{PS}$ a Phenol inactivated in PBS

Table IV A : Comparison of the potency of 2 suckling mouse brain vaccine (CVS Type) using nss and PBS as suspension media and inactivated by various methods.


Table IV $B$ : Comparison of the potency of 2 suckling mouse brain vaccine (pV-Type) using
NSS and PBS as suspension media and in activated : by various methods.


Remark : Virus Titre $=10^{-5.8}$


H-NS Heat inactivated in NSS
H-PS $=$ Heat inactivated in PBS
P-NS = Phenol inactivated in NSS
P-PS = Phenol inactivated in-PBS

Table VA : Comparison of the stability of 2 suckling mouse brain (CVS) by incubating at $37^{\circ} \mathrm{C}, 12$ Days (Accelerate degradation Test)


B-PS - Betapropiolactone inactivated in phosphate buffer saline (PBS) $\mathrm{H}-\mathrm{NS}=$ Heat inactivated $\ln \mathrm{NSS}$ |
H-PS $=$ Heat inactivated in PBS
P-NS = Phenol inactivated in NSS
P-PS $=$ Phenol inactivated in PBS

Table VB : Comparison of the stability of 2 suckling mouse brain (PV), by incubating at $37^{\circ} \mathrm{C}, 12$ Days (Accelerate degradation test)


Table VI Statistical Test in potency of semple vaccine.

| Method Suspension <br> of Inactivation | NSS | PBS | Total |
| :---: | :---: | :---: | :---: | :---: |
| BPL | $0.49,0.17,3.53,4.67,1.25$ | $0.44,0.16,3.45,5.13,1.07$ | 20.36 |
| Phenol | $0.27,0.07,0.64,0.23,0.24$ | $0.24,0.08,0.56,0.25,0.21$ | 2.97 |
| Heat | $0.40,0.13,2.68,2.72,0.48$ | $0.42,0.13,2.81,2.80,0.35$ | 12.92 |
| Total | 17.97 | 18.1 |  |


| Statistical Differenc | BPL VS Phenol VS Heat | BPL VS Phenol | Phenol VS Heat | Heat VS BPL | NSS | VS | PBS |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| F - Test | Significant At $0.05 \quad \mathrm{P} \quad 0.01$ | - | - | - | No Significant <br> At $P \quad 0.05$ |  |  |
| T - Test |  | Significant <br> At P 0.05 | $\begin{aligned} & \text { Signdificant } \\ & \text { At P } 0.05 \end{aligned}$ | $\begin{array}{ll} \text { Significant } \\ \text { At } P r & 0.05 \end{array}$ |  | - |  |

Table VII Statistical Test in potency of $2 \%$ SMB

| Suspension <br> Method <br> of Inactiva- <br> tion | NSS |
| :---: | :---: |
| BPL | $12.54 \times 10^{-2}$ |
| Phenol | $8.48 \times 10^{-2}$ |
| Heat | $10.43 \times 10^{-2}$ |
| Total | $64.36 \times 10$ |



Table VIII : Statistical test in the stability of $2 \%$ SMB (PV and CVS) in NSS and PBS as suspension media (by accelerate degradation test).



Table IX : Comparison of the potency of 3 lyophilized suckling brain vaccine (CVS)
in various stabilizers


Remark : Virus titre $=10^{-6.0}$

