received any vaccine within the previous 30 days (except tetanus toxoid and tetanus immune globulin); and with known allergy to the vaccine. Upon enrollment, the subjects underwent detailed history taking and thorough physical examination.

The subjects were then randomized into two groups. Group 1 (Pre-exposure) followed the standard ID regimen using a dose of 0.1 mL PVRV (Abhayrab) given intradermally on either deltoid on days 0,7 and 28. Group 2 (Post-exposure) followed the modified Thai Red Cross ID regimen (2-2-2-0-2) using a dose of 0.1 ml PVRV (Abhayrab) each given intradermally on both deltoids on days 0, 3, 7, and 28. Randomization was performed by taking one sealed envelop containing the randomly arranged grouping code. A nurse recorded the number allocated to the subject and the assigned code.

Vaccine and vaccination

The trial vaccine used, Abhayrab, is a purified vero cell based beta-propiolactone inactivated rabies vaccine manufactured by Human Biologicals Institute. The vaccines were from a common commercial lot (lot no: AYB1106) manufactured in May 2006. Each single dose vial contained lyophilized rabies antigens of strain L. Pasteur-2061/Vero with an NIH test potency of 5.56 IU. This was reconstituted to 0.5 mL with 0.9% sodium Chloride prior to injection. Each vial preparation also contained 0.015% thiomersol, maltose, and human serum albumin. The reconstituted vaccine from which each 0.1 mL ID dose was withdrawn was kept refrigerated, and was discarded 8 hours after reconstitution even if the entire 0.5 mL content had not been fully consumed. A nurse administered the vaccine intradermally following the WHO recommendation [8]. A one-milliliter syringe fitted with a 26-guage needle was used in administering the vaccine.

Vaccination safety evaluation

After vaccination, the subjects were asked to stay for 30 minutes and were evaluated for any occurrence of adverse reactions/events as defined in **Table 1.**

The subjects were asked to return for subsequent doses of the vaccine and for blood extraction. At each visit, the subjects were asked about any adverse reactions they personally noted. Efforts were made to ensure that the subjects returned for follow-up doses and examination, including conveying reminders by phone and/ or home visits.

Table 1. The severity scale used for evaluating the adverse reactions observed after the ID vaccinations

Local reactions	**1=Mild**	**2=Moderate**	**3=Severe**
Pain	Reacts when site is touched	Cries when site is touched	Cries when limb is moved
Redness, swelling Induration/unsolicited local reactions	Largest diameter of the reaction < 2.5cm	Largest diameter of the reaction between 2.5-5.0 cm	Largest diameter of the reaction > 5.0 cm
Itchiness	Easily tolerated	Sufficiently discomforting to interfere with daily activity	Preventing normal daily activity
Systemic reactions			
Fever	37.5-38.0°C	38.1-39.5°C	≥ 39.6°C
Malaise, headache, rash, myalgia, GI upset	Easily tolerated	Sufficiently discomforting To interfere with daily activity	Preventing normal daily activity

Serum sample collection and antibody assay

Four mililiters of blood from each subject were drawn on days 0 (pre-vaccination sample), 14 and 28. The schedule for blood sampling in some subjects deviated by ±1 day for the day 14 samples and ± 3 days for day 28 samples.

Blood samples were drawn by a medical technologist using plain vacutainer tubes. A label was affixed onto the vacutainer tube immediately prior to blood sample drawing. The collected blood samples were left at room temperature for 30 minutes to two hours for clotting and then centrifuged at 3000 rpm for 5 to 10 minutes to separate the sera. The Serum samples were aliquoted and coded, and kept frozen at -20 to - 80°C throughout the study period. The temperatures of the freezers were monitored and documented during the entire study period. The coded serum samples were dispatched to the Rabies Research Laboratory of the Research Institute for Tropical Medicine, Department of Health, Alabang, Muntinlupa City for determination of neutralizing antibody titers by the Rapid Fluorescent Focus Inhibition Test (RFFIT) following the Centers for Disease Control and Prevention (CDC) protocol. Neutralizing antibody titers were expressed in IU per mL. Subjects with antibody titers 0.5 IU per mL were considered to have sero converted. Such

seroconversions served as the primary evaluation criteria for immunogenicity, while the Geometric Mean Concentrations (GMC) derived from the logarithmic transformation of the antibody titers obtained on days 7, 14 and 28 served as the secondary quantitative evaluation criteria. The significance of the GMC values was assessed in relation to their 95 percent confidence itnervals (95%CI).

Subject withdrawal procedure

When a subject failed to appear for a follow-up examination, efforts were undertaken to locate and determine the subject's health status. Subjects who were unable to return within the allowable time frame still completed their vaccination but were excluded from the immunogenicity analysis. However, their safety evaluation data were still considered. The subjects who decided to withdraw from the study were allowed to do so, but it was ascertained that the withdrawal was not due to an adverse event. The reasons for withdrawal were noted in the CRF.

Data analysis

Data recorded in the individual CRFs were encoded and analyzed using EPI-INFO version 6.0, Centers for Disease Control, USA.

Results

One hundred forty nine subjects were enrolled in this clinical trial, 73 in the pre-exposure group (Group 1) and 76 in the post-exposure group (Group 2). Eventually, 120 subjects (80%), 60 in each group, completed the day 28 ID immunization and were included in the According -to-protocol (ATP) analysis of immunogenicity. Twenty-nine subjects were withdrawn from the study mainly because of conflict with their guardian's work schedule. Three subjects withdrew because of failure to submit to blood extraction. However, the safety data from all the 149 immunized subjects on day 0 and those that could be obtained from days 3 and 7 were included in the intent to treat analysis.

The safety evaluation data is presented in **Table 2.** Mild local reactions, particularly redness and itchiness were observed in varying frequencies (4.0-63.0%) among the subjects in both groups throughout the evaluations. Only one subject, at most, experienced mild pain at any evaluation point. No moderate or severe reactions were observed or reported by any of the subjects, including those who dropped out. Mild fever was observed in only one subject in Group 1 and two in Group 2, and it occured only in association with the first day (day 0) of vaccine administration. No other systemic reaction was noted in any of the subjects during any of the evaluation points. There was no indication for administering treatment for any of the reactions noted.

All the 120 subjects included in the ATP analysis for immunogenicity demonstrated seroconversions (antibody titers were greater than the WHO cut-off level of 0.5 IU/mL) on days 14 and 28 **(Table 3).** Thus, the seroconversion rates for both groups were 100% as applicable to these 120 subjects. Three subjects in Group I presented values greater than 60 IU/mL. The GMC values for Group 1 were 3.30 IU/mL and 4.37 IU/mL on days 14 and 28 respectively. While those of Group 2 were 3.73 IU/mL and 4.82 IU/mL, respectively. These GMC values were within their 95% CI's **(Table 4).**

Table 2. The number and percentage (%) of subjects with mild reactions in the PVRV pre-and post-exposure ID regimen groups

Reactions	**Pre-exposure group**			**Post-exposure group**			
	Day 0	Day 7	Day 28	Day 0	Day 3	Day 7	Day 28
Starting N=149 (73/76)	N=73	N=67	N=60	N=76	N=63	N=63	N=60
Local reactions (injection site)							
Redness	28(38.4%)	6(9.0%)	13(21.7%)	3.(3.9%)	40(63.5%)	10(15.9%)	20(33.3%)
Itchiness	15(20.5%)	16(23.9%)	3(5.0%)	8(10.5%)	12(19.0%)	21(33.3%)	3(5.0%)
Pain	1(1%)	1(1.5%)	0	0	1(1.6%)	1(1.6%)	0
Systemic reactions							
Fever	1(1.4%)	0	0	2(2.6%)	0	0	0
Others	0	0	0	0	0	0	0