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Production and evaluation of a chromatographically purified Vero cell rabies vaccine (PVRV) in China using microcarrier technology

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Abbreviations: ATCC, American Type Culture Collection; CVS, challenge rabies virus; FDA, Food and Drug Administration; GMT, geometric mean titer; MIT, mouse inoculation test; PHKCV, primary hamster kidney cell culture vaccine; PVRV, purified Vero cell rabies vaccine; PCECV, purified chick embryo cell rabies vaccine; RVNA, rabies virus neutralizing antibody; RFFIT, rapid fluorescent focus inhibition test; WHO, World Health Organization

China is a high population country with millions of animal bite cases every year; thus, it is necessary to explore and develop more effective and productive rabies vaccines for human use. To establish a safe, effective, inexpensive and high-yield rabies vaccine, a non-adjuvant purified Vero cell rabies vaccine produced in the SPEEDA PVRV microcarrier bioreactor was developed by Liaoning Chengda Biology Co. Ltd. in China. This vaccine was produced using Vero cells that were cultured in a microcarrier bioreactor. A microcarrier bioreactor containing 25 g/L of Cytodex-1 was used for perfusion culture. The Vero cell culture density was up to 1.2–1.5 x 10^7 cells/ml, viruses could be constantly harvested for 18–22 days, and the resulting vaccine immunizing potency was ≥ 4.5 IU/ml. Vaccine safety and immunogenicity post-immunization were also assessed. A total of 602 volunteers were enrolled and divided into two groups that were vaccinated with either SPEEDA PVRV or VERORAB PVRV on days 0, 3, 7, 14 and 28. All subjects vaccinated with SPEEDA PVRV showed no serious local or systemic adverse effects. The positive conversion rate of serum neutralizing antibodies against the rabies virus reached 100% in both the test and control groups (inoculated with VERORAB PVRV) at 14 days and 45 days after vaccination, and no significant difference was found between the neutralizing antibody geometric mean titers (GMTs) of the two groups. SPEEDA PVRV is appropriate for mass production and shows satisfactory clinical safety and immunogenicity for human post-exposure prophylaxis of rabies.

Introduction

Rabies, one of the major zoonotic diseases caused by the rabies virus, is almost always 100% fatal once symptoms occur. Pre- or post-exposure rabies vaccination is the only effective way to prevent human rabies. In China, more than 2,000 rabies cases occur each year, and 87.5% of these cases are caused by dog bites.[1] Moreover, over 12 million persons are inoculated with the rabies vaccine for pre- and post-exposure prophylaxis every year.[2]

Prior to 1980, the rabies vaccine for human use was produced in China from sheep brain tissue, which induced severe adverse effects, such as neuroparalysis and encephalomyelitis, and its immunogenicity was not entirely satisfactory. In 1980, China abandoned animal brain tissue vaccine production in favor of cell culture vaccine production. Presently, two types of cell culture human rabies vaccines are produced in China: primary hamster kidney cell culture vaccine (PHKCV) and Vero cell rabies vaccine (PVRV).[3] Currently, cell culture rabies vaccines are produced using the traditional roller bottle method by most manufacturers in China. The roller bottle requires more labor and a larger space, provides a small surface area for cell growth per unit volume, shows a low cell density, and limits cell culture monitoring. Thus, vaccine production using the traditional roller bottle leads to a low yield and low effectiveness. Due to the high incidence of rabies and the large dosages needed for pre- or post-exposure rabies vaccination due to the high population in China, the need to explore and develop more effective and productive rabies vaccines for human use is urgent.

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Table 1. Comparison of cell density, virus titer, and harvest time among different volumes of microcarriers

Microcarrier density (g/L)	Primary cell density (cells/ml)	Final cell density (cells/ml)	Highest viral titer (LD_{50}/ml)	Average viral titer (LD_{50}/ml)	Harvest time (d)
5	0.3×10^6	0.3×10^7	7.2	6.7	13
15	0.8×10^6	0.8×10^7	7.6	7.0	19
20	1.2×10^6	1.2×10^7	8.0	7.2	18–22
25	1.5×10^6	1.5×10^7	8.5	7.6	22–24

Table 2. Quality indicators in three batches of purified intermediate SPEEDA PVRV

Sample number	Protein level before purification (μg/g)	Protein level after purification (μg/g)	Impurity protein removal (%)	Glyco-protein recovery (%)	Residual DNA level (pg/0.5 ml)	Residual bovine serum albumin (ng/0.5 ml)	Potency (IU/0.5 ml)
20030301	14600	75	99.95	79.5	< 10	< 25.0	5.70
20030302	15100	72	99.95	80.1	< 10	< 12.5	7.01
20030303	15900	73	99.95	79.7	< 10	< 25.0	6.02

In 2002, microcarrier bioreactor cell culture technology was introduced by Liaoning Chengda Biology Co. Ltd. in China from Bari Consultants LLC Company in Florida. Based on the digestion, absorption, and innovation of the microcarrier technology, SPEEDA-purified Vero cell rabies vaccine (SPEEDA PVRV) was developed using Vero cells and the rabies virus of the Pasteur vaccine strain PV2061. SPEEDA PVRV was the first purified Vero cell rabies vaccine for human use based on the bioreactor technology in China. Since it was approved by the China Food and Drug Administration (FDA) in 2005, the production process had not been changed. To date, it has been used for nearly 7 years since the vaccine was developed, and a total of 25 million people have received this vaccine in China and some other countries where SPEED PVRV had been registered including India, Thailand, Uzbekistan, Kyrgyzstan, Egypt, Malawi and other 20 countries. Clinical observations of vaccination results indicate that the vaccine shows satisfactory safety and immunogenicity. The present study summarizes the production techniques and efficacy evaluation of the vaccine usage in China over the past 7 years.

Results

Cell culture and virus amplification. The cell density could be enhanced by increasing the concentration of the microcarrier, which resulted in the varying virus titers and harvesting frequencies. At a microcarrier concentration of 25 g/L, which was chosen for the production, the cell density reached 1.5×10^7 cells/ml, which was five times as much as the cell density on 5 g/L of microcarriers, 1.88 times as much as that on 15 g/L of microcarriers, respectively, and slightly higher than that on 20 g/L of microcarriers. The average virus titer harvested on 25 g/L of microcarriers was 7.6 log LD_{50}/ml, being 0.9 log LD_{50}/ml higher than that on 5 g/L of microcarriers, 0.6 log LD_{50}/ml higher than that on 15 g/L of microcarriers and 0.4 log LD_{50}/ml higher than that on 20 g/L of microcarriers, respectively. The effective harvest time of the viruses using 25 g/L of microcarriers was delayed 9–11 d as compared with that using 5 g/L of microcarriers, which was delayed 3–5 d compared with 15 g/L of microcarriers, and were almost equal to 20 g/L of microcarriers (**Table 1**).

Virus titer and harvest time. The virus titer and harvest time using two different cell culture processes (bioreactor and roller bottle) showed that at a microcarrier concentration of 25 g/ L, the highest virus titer was up to 8.5 log LD_{50}/ml, the mean titer was 7.6 log LD_{50}/ml, and the effective harvest period was 22–24 d at intervals approximately 10–12 times. Using a roller bottle, the highest virus titer reached 6.5 log LD_{50}/ml at 96 h post-infection, the effective harvest was approximately 13–15 d at intervals about four times normally.

Virus purification. The continuously harvested virus was concentrated by ultrafiltration and chromatographic purification was then conducted with chromatography media using the Sepharose gel system. Three batches of purified intermediate SPEEDA PVRV (lots 20030301, 20030302 and 20030303) were subjected to quality testing; the protein level before and after purification, residual DNA levels, residual bovine serum albumin levels, glycoprotein recovery, and potency were determined (**Table 2**).

SPEEDA PVRV quality testing. Sterilization and inactivation of SPEEDA PVRV were performed according to the Pharmacopoeia of the People's Republic of China (the ninth edition issued in 2010). All quality test results were equal to or better than the national standard of rabies vaccine quality for human use. Specifically, the potency of SPEEDA PVRV was ≥ 4.5 IU/dose, which is much higher than the national standard (2.5 IU/dose) (**Table 3**).

Safty of SPEEDA PVRV. Close observations for local and systemic adverse reactions in volunteers at 30 min, 4, 24, 48 and 72 h after immunization showed no immediate reaction. Local reactions were mainly occurred after the 1st and 2nd dose injection, scarcely observed after the 3rd dose injection and none after the 4th and 5th dose injection. Local reactions were presented as itching and pain at the inoculation site. The local reaction rate of the test group was 0.65% and that of the control group was 0.43%; no significant difference was found between the two groups (χ^2 = 0.6054; $p > 0.05$). Observations for systemic reactions in the two

Table 3. Quality of SPEEDA PVRV according to the national standard of rabies vaccine for human use

Items	National standard of rabies vaccine for human use	SPEEDA ® PVRV
Sterility test	Negative (-)	Negative (-)
Residual bovine serum albumin	≤ 50 ng/dose	≤ 50 ng/dose
Potency test	≥ 2.5 IU/dose	≥ 4.5 IU/dose
Residual Vero cell DNA	≤ 100 pg/dose	≤ 100 pg/dose
Bacterial endotoxin	≤ 50 EU/dose	≤ 50 EU/dose
Total protein	≤ 80 μg/dose	≤ 80 μg/dose
Host protein	≤ 4 μg/dose	≤ 4 μg/dose
Antibiotics	≤ 50 ng/dose	-
pH	7.2–8.0	7.2–8.0

Table 4. Local reactions of the control and test groups after vaccination

Group	Injection	Observed subjects	Pain, itching		Swelling		Induration		Total	
			number	%	number	%	number	%	number	%
Test group	1	502	8	1.59	2	0.40	0	0	10	2.0
	2	467	2	0.43	1	0.21	0	0	3	0.64
	3	452	1	0.22	1	0.22	0	0	2	0.44
	4	443	0	0	0	0	0	0	0	0
	5	432	0	0	0	0	0	0	0	0
	Total	**2296**	**11**	**0.48**	**4**	**0.17**	**0**	**0**	**15**	**0.65**
Control group	1	100	1	1.0	0	0	1	1.0	2	2.0
	2	97	0	0	0	0	0	0	0	0
	3	93	0	0	0	0	0	0	0	0
	4	85	0	0	0	0	0	0	0	0
	5	85	0	0	0	0	0	0	0	0
	Total	**460**	**1**	**0.23**	**0**	**0**	**1**	**0.22**	**2**	**0.43**

groups showed that a few people presented with transient fever (37.1–38.6°C) after the first dose, but no serious systemic adverse reaction was observed. All subjects presenting with adverse reactions received no medical intervention. Adverse reactions are summarized in **Table 4** (local reactions) and **Table 5** (abnormal reaction rate of temperature).

Serologic evaluation of SPEEDA PVRV. Fifty subjects in the test group and 35 in the control group were randomly selected for serum neutralization antibody testing. The results showed that neutralizing antibody positive conversion rate were 0 at 0 d (virus neutralizing antibody (RVNA) concentration was less than 0.2 IU/ml), and the neutralizing antibody positive conversion rates were 100% at 14 d and 45 d when the RVNA concentrations were 0.8–11.9 IU/ml and 2.7–29.2 IU/ml respectively. The specific RVNA concentration and RVNA geometric mean titer (GMT) was shown in **Table 6**.

Discussion

Presently, cell culture rabies vaccines used in China are mainly the primary hamster kidney cell culture vaccine (PHKCV) and the purified Vero cell rabies vaccine (PVRV), which are produced mostly in roller bottles. This method leads to low yields and it is often difficult to guarantee quality and consistency. Microcarrier bioreactor cell culture shows larger yields, fewer differences among batches, and more viral antigens are harvested.

The high-density cells in the bioreactor are cultured by continuous perfusion. Viruses are continuously harvested so that the bioreactor can always maintain a nutrition environment for cell growth, thus avoiding the accumulation of products resulting from high-density cell metabolism. During the entire procedure, completely closed pipe production processes and automated monitoring technologies are used to reduce labor input and simplify manual processes, as well as significantly reduce the cost as compared with vaccines produced by other cell culture methods.

Perfusion bioreactor technology can improve the density of cell growth. High-density viruses can be harvested in purified rabies vaccines produced using the microcarrier bioreactor culture technique and downstream purification process. In the current study, the potencies of three batches of semi-products were 5.70, 7.01 and 6.02 IU/0.5 ml, respectively. The products met the quality standards of the WHO, similar to the international advanced Vero cell rabies vaccines.[12,13]

Tests aimed at determining the safety and immunogenicity of SPEEDA PVRV revealed no severe systemic adverse effects. Local reactions were mainly manifested by pain and itching at

Table 5. Abnormal reaction rate of temperature in the control and test groups after vaccination

Group	Injection	Observed subjects	Weak reaction		Moderate reaction		Strong reaction		Total	
			number	%	number	%	number	%	number	%
Test group	1	502	1	0.19	4	0.8	0	0	5	1.0
Control group	1	100	0	0	1	1.0	0	0	1	1.0

Table 6. Comparison of antibody conversion and geometric mean titer (GMT) between the test and control groups after vaccination

Group	0 d			14 d			45 d		
	Neutralizing antibody (IU/ml)	GMT (IU/ml)	Positive rate (%)	Neutralizing antibody (IU/ml)	GMT (IU/ml)	Positive rate (%)	Neutralizing antibody (IU/ml)	GMT (IU/ml)	Positive rate (%)
Test group	≤ 0.2	≤ 0.2	0	0.8–11.9	5.2	100	2.7–29.2	9.5	100
Control group	≤ 0.2	≤ 0.2	0	0.9–12.7	5.6	100	1.5–25.4	9.8	100

the injection site, local reaction rates observed in the test group and control group were 0.62% and 0.43% respectively; systemic side effect rates occurred in the test group and control group were 1% and 1% respectively, no significant differences were found in these two groups. These results suggested that SPEEDA PVRV and VERORAB PVRV showed similar safety.

The positive conversion rate of neutralizing antibodies reached 100% at 14 d and 45 d post-vaccination. GMTs in the test and control groups were not significantly different and show that SPEEDA PVRV and VERORAB PVRV have the same immunogenicity. Taken together, SPEEDA PVRV shows good immunogenicity after post-exposure immunization.

To date, SPEEDA PVRV has been used in 25 million people, not only in China, but also in other Central European and African countries. Under the Essen 5-dose regimen, the neutralizing antibody positive conversion rate of SPEEDA was 100% after 14 d, and the seroconversion rates at 180 d and 360 d were 100% and 89.1% respectively, which indicated that PEEDA PVRV possessed the persistent immunogenicity.[14]

In 2011, SPEEDA PVRV was approved in the 2-1-1 immunization program by the China FDA. The immunogenicity and safety of SPEEDA PVRV was also compared with that of Rabipur PCECV (Novartis-Behring, Germany) under 2-1-1 program. The seroconversion rate of the subjects immunized with SPEEDA and Rabipur were 69.39% and 68.75% respectively on day 7 after vaccination, and were both 100% on day 14. Meanwhile, both vaccines appeared safe and were well tolerated by the subjects. All the above showed that PVRV (SPEEDA), under 2-1-1 regimen, is equally safe and immunogenic as the PCECV (Rabipur) for post-exposure prophylaxis vaccination.[15]

In China, rabies occurs most commonly in rural and poor areas and most of the human rabies cases were famers. Because of the high price and shortage sometime of the rabies vaccine, many people in these areas cannot receive timely post-exposure immunization and may acquire rabies. SPEEDA PVRV is relatively cheaper than the imported human rabies vaccine (32RMB/dose for SPEEDA, 78 RMB/dose for Rabipur), which makes it easier to afford for most people in the high epidemic but low economic development areas.

WHO recommends that rabies virus vaccine antigen concentrations should not be less than 2.5 IU/dose, and the serum neutralizing antibody titer post-vaccination must be more than 0.5 IU/ml.[16-18] SPEEDA PVRV is produced using microcarrier bioreactor technology. Our study shows that the safety and immunogenicity of SPEEDA PVRV equal to similarly imported PVRVs. Because of the low price and large yields, SPEEDA PVRV will offer great support for rabies control and prevention.

Materials and Methods

Cell culture. Vero cells at passage 134 were obtained from the American Type Culture Collection (ATCC; 1507 1630 1758).[4] Bioreactors (30 L each, Bioflo 4500 Fermentor/Bioreactor) were purchased from New Brunswick Scientific Co. Ltd. Cytodex-1 was purchased from GE Healthcare Company.[5,6]

Vero cells at passage 138 from the cell bank were grown separately using culture medium containing 10% calf serum in a culture flask. After growing the cells into a monolayer, they were subjected to further passages to passage 141. When the cell density was up to 1.0–1.2 × 10^6 cells/ml, they were transferred into a bioreactor containing 25 g/L of Cytodex-1 for perfusion culture. The cells cultured in the bioreactor could not be used beyond passage 143.

Virus strain and amplification. The PV2061 strain of Pasteur obtained from the United States Centers for Disease Control and Prevention was used for vaccine production. Vero Cells cultured at 37°C for 5 d were infected with the PV2061 virus when the density of the cells reached 1.2–1.5 × 10^7 cells/ml. The temperature in the bioreactor was then changed to 33–35°C and culture medium containing 1% calf serum was used for perfusion culture. Viruses were harvested after 3 d of culture and could be harvested continuously for 18–22 d.

Virus was also cultured in a traditional roller bottle. Two liters of Vero cells with a density of 3 × 10^4 cells/ml were transferred into a 15-L roller bottle and cultured for 3 d. Subsequently, they were infected with the PV2061 virus strain. The virus was harvested after 3 d of culture and could be harvested continuously for 13–15 d. The titers of viruses cultured in the roller bottle and bioreactors were determined.

Preparation and quality control of SPEEDA PVRV. Viruses were harvested at 2-day intervals until the 18th–22nd day, and viral titers were determined using the mouse inoculation test (MIT). The harvested virus was concentrated about 20 times using a clarification filtration and ultrafiltration concentration pipeline in a whole-course aseptic connection, and was then inactivated using a 1/4,000 dilution of β-propiolactone at 4°C. After passing test examinations such as virus titration, sterility testing, mycoplasma testing, and inactivation efficacy testing, chromatographic purification was conducted to remove the residual bovine serum, cellular debris, residual cell DNA, and other impurities.[7] Comprehensive testing was performed in accordance with the standards provided in the "Pharmacopoeia of the People's Republic of China" (the eighth edition issued in 2005); thus, SPEEDA PVRV meets the standards of the national requirement for the human rabies vaccine and was approved by the China FDA in 2005.[4]

Observed subjects. Based on the principle of informed consent and voluntary participation, we selected 602 male and female post-exposure volunteers who were mostly bitten by dogs under category II exposure and did not need to inject human rabies immunoglobulin. All the volunteers were 10 to 60 y old, were in good health condition, and had no history of rabies vaccination and inoculation contraindications. Inquiry and health checks were conducted before vaccination. Volunteers who met the inclusion criteria were divided into two groups: a test group (n = 502) and control group (n = 100).

Vaccination schedule. The test group was inoculated with SPEEDA PVRV (lot 20030301-6; Liaoning, China) with a potency of 6 IU per dose (0.5 ml). The control group received the purified human Vero cell rabies vaccine imported from France [VERORAB PVRV, lot U1594; manufactured by Sanofi Pasteur (Lyon, France)] with a potency of 6 IU per dose (0.5 ml). The potencies for both of the vaccines were determined by NIH mouse potency test.

Both the test and control groups were subjected to the same immunization schedule—five separate doses by upper arm deltoid intramuscular injection on day 0, 3, 7, 14 and 28—which is one of the post-exposure schedules recommended by the World Health Organization (WHO).[8,9]

Clinical observation. Clinical symptoms were mainly observed in both the test and control groups after each vaccination, and systemic and local reactions of each volunteer were observed within 30 min and at 4, 24, 48, and 72 h. Systemic reactions mainly referred to body temperature, subjective symptoms, and objective signs such as fever, headache and malaise. Local reactions were mainly manifested by local swelling, pain, suppuration and induration at the injection site.

Exclusion criteria of the observed subjects were as follows: patients with acute febrile illness (axillary temperature > 37.5°C); patients with immune deficiency syndrome; patients who were receiving immunosuppressive therapy; patients with a history of vaccination with rabies vaccine or passive immunosuppressants (anti-rabies serum or rabies immune globulin); patients who showed rabies symptoms; those who participated in another clinical trial and underwent an alternative vaccine inoculation simultaneously.

Serology evaluation. Three milliliters of whole blood specimens were collected on day 0, 14 and 45 after the first dose injection respectively from 50 subjects in the test group and 35 subjects in the control group. The serum samples were isolated from the collected blood specimens and stored below -20°C. All analysis was conducted at the National Institute for Viral Disease Control and Prevention, the Chinese Center for Disease Control and Prevention. Rabies virus neutralizing antibody levels were measured using the rapid fluorescent focus inhibition test (RFFIT), as recommended by the WHO.[10] Briefly, a constant dose of a previously titrated, cell culture-adapted, challenge rabies virus (CVS strain) was incubated with a serial dilution of the sera to be titrated. A reference serum (30 IU/ml) purchased from the National Institute for Biological Standards and Control (UK) of known titer was included in each test. After 1 h of incubation at 37°C, BSR cells (clone BHK-21) were added in each well. After 24 h of incubation at 37°C in 5% CO_2, the percentage of infected cells for each sera dilution were estimated, which allows for titer determination of the unknown neutralization antibody by comparison with the reference serum. The titer of the neutralization antibody from the sera was recorded as International Units per milliliter (IU/ml), which is identified worldwide. Reaching adequate RVNA concentrations of 0.5 IU/ml was defined as seroconversion based on the WHO criteria and considered to be positive.[11]

Statistical analysis. SPSS 11.5 software was used for statistical analysis. A χ^2 test was used to compare the difference between local reaction rates in the two test groups. A *P*-value less than 0.05 was deemed to indicate a statistically significant difference.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Cui-ping Y, Hang Z, Hui W, Xinxin S, Lihua W, Wenwu Y, et al. Epidemiological analysis of rabies in 2010, China. Chinese Journal of Experimental and Clinical Virology 2011; 25:434-6.
2. Situation of rabies prevention and control in China. Ministry of Health of the People's Republic of China 2009.
3. Yongxin Y. Development of the current situation of the domestic and foreign rabies vaccine. Shanghai Journal of Preventive Medicine 2006; 18:216-8.
4. Li C, Jun G, Jianying H, Zhumu B, Deming Y, Junwei Y, et al. Preparation of Rabies Vaccine for Human Use by Cell Culture in Bioreactor. Chinese Journal of Biologicals 2006; 19:288-90.
5. Cytodex™ 1: Beaded microcarriers for cell culture, Pharmacia Fine Chemicals, Technical Booklet Series, Uppsala, Sweden 1978.

6. Kruse PF Patterson MK. Tissue culture, methods and applications. New York: Academic Press, 1973; 372-377.
7. van Wezel AL, van Herwaarden JA, van de Heuvel-de Rijk EW. Large-scale concentration and purification of virus suspension from microcarrier culture for the preparation of inactivated virus vaccines. Dev Biol Stand 1979; 42:65-9; PMID:223923.
8. Yan-ping L, Li C, Rong-cheng L, Jiongxian T, Yi N, Fusheng C, et al. Safety and immunogenicity of a new inactivated rabies vaccine (Vero Cell) in healthy adult. Guangxi Prev Med 2005; 11:193-5.
9. WHO expert consultation on rabies: first report. WHO technical report series 931. Geneva: WHO; 2004.
10. Smith JS, Yager PA, Baer GM. A rapid reproducible test for determining rabies neutralizing antibody. Bull World Health Organ 1973; 48:535-41; PMID:4544144.
11. WHO expert consultation on rabies, 1st report. Geneva 2005; 88.
12. WHO tech Rep Ser No. 700, 1984.
13. WHO tech Rep Ser No. 747, 1987.
14. Wang LY, Sun MP, Zhang XC, Suo LD, Xu RH, Zou YJ, et al. Safety and immunogenicity of two freeze-dried Vero cell rabies vaccines for human use in post-exposure prophylaxis. Vaccine 2011; 29:2679-81; PMID:21296694; http://dx.doi.org/10.1016/j.vaccine.2011.01.053.
15. Liu H, Huang G, Tang Q, Li J, Cao S, Fu C, et al. The immunogenicity and safety of vaccination with purified Vero cell rabies vaccine (PVRV) in China under a 2-1-1 regimen. Hum Vaccin 2011; 7:220-4; PMID:21311216; http://dx.doi.org/10.4161/hv.7.2.14003.
16. Meslin FX, Kaplan MM, Koprowski H. Laboratory techniques in rabies. 4th edition. Geneva: World Health Organization, 1996: 7.
17. Fishbein DB, Yenne KM, Dreesen DW, Teplis CF, Mehta N, Briggs DJ. Risk factors for systemic hypersensitivity reactions after booster vaccinations with human diploid cell rabies vaccine: a nationwide prospective study. Vaccine 1993; 11:1390-4; PMID:8310759; http://dx.doi.org/10.1016/0264-410X(93)90167-V.
18. Suntharasamai P, Chaiprasithikul P, Wasi C, Supanaranond W, Auewarakul P, Chanthavanich P, et al. A simplified and economical intradermal regimen of purified chick embryo cell rabies vaccine for postexposure prophylaxis. Vaccine 1994; 12:508-12; PMID:8036824; http://dx.doi.org/10.1016/0264-410X(94)90308-5.