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Evaluation and Validation of Potency Testing Method for Live Rubella Virus Vaccine

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ABSTRACT

The potency of live rubella virus vaccine is usually determined by the microtitration method using *in vitro* cytopathic effect (CPE) with rabbit kidney epithelial (RK-13) cell culture. However, it is difficult to identify the rubella viral CPE through microscopic examination. Therefore, developing a validated and accurate method for potency determination is important for the laboratories of the government and vaccine manufacturers to ensure the effectiveness of rubella vaccination. In this study, we evaluated different culture conditions of RK-13 cells for the potency test of live rubella virus vaccine. We found that the RK-13 cells with the initial plating number of 13,000 per well of 96-well cell culture plate, supplied with 2% fetal bovine serum (FBS) could form and maintain a normal monolayer for 14 days when incubated at 33˚C. In addition, our results showed that the rubella viral CPEs are developed faster and simpler under 2% of serum concentration and at 33˚C. The results of validation analysis finally confirmed that the culture condition with plating number of 13,000 cells/well, 2% of serum concentration, and 33˚C incubating temperature achieve the most accurate and precise results for the CPE potency test of live rubella virus vaccine.

Key words: cytopathic effect, live rubella vaccine, accuracy, precision and validation

INTRODUCTION

For many years, the live rubella virus vaccine has been developed to protect people from rubella and congenital rubella syndrome (CRS). The World Health Organization (WHO) requirements for rubella vaccine were first formulated in 1976[1]. Currently, the production of this vaccine is well established with the live attenuated virus strains. These attenuated strains, including Cendehill, HPV-77, Takahashi, Matsuura, and RA27/3, were initially licensed from 1969 to 1970[2]. After a long term evaluation for vaccine safety and efficacy, RA27/3 and Cendehill were recommended for use, for the attenuated strains of live rubella vaccine and these two kinds of rubella vaccines are widely available throughout the world. The RA27/3 strain of rubella virus has been used since 1965. Compared with others, the RA27/3 strain developed by Dr. S. A. Plotkin[3,4] was attenuated by additional passages of cold adaptation in suitable cell cultures[5]. The final passages of cold adaptation were made first at 35˚C, then at 33˚C, and finally at 30˚C. In addition, the results from evaluation of vaccine efficacy showed that the vaccine recipients could maintain the antibody titer against rubella virus in their sera for 16-17 years after vaccination[5].

In Taiwan, the initially used live rubella virus vaccine was RUDIVAX® (manufactured by Pasteur Merieux, France) and it was licensed in 1976. There are five similar products currently available on the Taiwan market (Table 1). These vaccines are all manufactured with live attenuated virus of the RA27/3 strain and harvested from MRC-5 or WI-38 human fibroblast cell cultures. Live rubella virus vaccine was widely introduced to Taiwan's junior high school girls in 1984, and there were rare reported incidences of rubella during the period of 1988 to 1993[6]. Additionally, to ensure the vaccine efficacy against rubella, poliomyelitis, measles and other infectious diseases in Taiwan, the National Laboratories of Foods and Drugs (NLFD) of the Department of Health (DOH) needs to safeguard the vaccine potency through the lot-by-lot release process[6]. For this reason, an accurate and precise potency testing method is very important for evaluating the efficacy of live rubella virus vaccine.

A wide variety of different testing methods can be used to quantify the virus content of live virus vaccine. There are two main *in vitro* testing methods, the plaque formation unit (PFU) and the CPE methods, for vaccine potency determination and validation[7]. The introduction of the CPE method with RK-13 cells for quantifying the rubella vaccine potency usually made it possible to obtain more reliable results than that of the PFU method in most situations[8, 9]. However, the CPE method still has some accuracy and precision problems during the potency test[10-12]. The major concern is that it is not easy to identify the rubella viral CPE through microscopic examination. Plotkin and Beale indicated that rubella CPE is either absent or mild under several culture systems[13]. Furthermore, we found the RA27/3 viral CPE looks like the morphology of cell over-growth under the RK-13 cell culture system. It is easy to make a mistake on rubella viral CPE identification and raises the bias of potency results. Although the vesicular somatitis virus (VSV) interference test has been

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Table 1. The currently available live rubella virus vaccines in Taiwan

Manufactures	Product	Virus Strain	Cell Substrate	Year Licensed
Pasteur Murieux Serum & Vaccines, France	RUDIVAX®	RA27/3	MRC-5	1976
Merck & Co., Inc., United States	MERUVAX II®	RA27/3	WI-38	1982
Swiss Serum and Vaccine Institute Berne, Switzerland	RUBEATEN® BERNA	RA27/3	MRC-5	1982
SmithKline Beecham Biologicals, Belgium	ERVEVAX®	RA27/3	MRC-5	1989
The Research Foundation for Microbial Disease of Osaka, Japan	Rubella Virus Vaccine Live, Attenuated "BIKEN"	RA27/3	WI-38	1995

introduced to assist in recognizing the rubella viral CPE, this interference method is more complicated and needs more long-term incubation to obtain the results[14, 15]. Most importantly, the VSV is classified into Biosafety Level 3 (BSL-3) by the Centers for Disease Control and Prevention, U. S. A. and should be handled under BSL-3 practices and facilities[16]. Even a number of laboratory-adapted strains (Indina, San Juan or Glascow) should be dealed with properly with BSL-2 practices and facilities. Therefore, developing a faster, safer, and more precise method is very important for potency evaluation of live rubella virus vaccine.

In this study, we compared and evaluated different culture conditions of RK-13 cells for the potency test of live rubella virus vaccine. We found that the RK-13 monolayer supplemented with 2% FBS at 33˚C can maintain the normal morphology during the testing period of 14 days. The evaluating results showed rubella viral CPE can be acquired faster and more accurate at 33˚C incubation than at other temperature conditions. The results of validation analysis also confirmed that the 33˚C incubation can contribute the CPE potency test of live rubella virus vaccine to achieve the most accurate and precise results.

MATERIALS AND METHODS

I. *Materials*

Minimum essential medium (MEM) containing Earle's salts, N-acetyl-L-alanyl-L-glutamine (Ac-ala-gln), and sodium bicarbonate and antibiotics (100x, lyophilized) were purchased from BIOCHROM KG (Berlin, German). Heat-inactivated and qualified fetal bovine serum (FBS) and trypsin/ethylenediaminetetraacetic acid (trypsin /EDTA) solution were purchased from Gibco BRL (Grand Island, NY, USA). The ISOTONE II azide free balanced electrolyte solution was supplied from Beckman Coulte, Inc. (Miami, FL, USA). The 96-well cell culture plates, tissue culture flasks, and other plastic accessories were from Corning/Costar (Nagog Park Action, MA, USA). The VSV (ATCC VR-158) was purchased from the American Tissue Culture Collection (ATCC). The live rubella virus vaccines were obtained from the Center for Disease Control, Department of Health, Taiwan, R.O.C.

II. *Cell Culture and Cell Growth Test*

RK-13 cells (ATCC CCL-37) were purchased from ATCC. The master cell bank (MCB) and working cell bank (WCB) of RK-13 cells were established for routine potency test. RK-13 cells were cultured in MEM supplemented with 5% FBS in a humidified incubator at 37˚C under 5% CO_2 condition. The cells were subcultured with 1 x trypsin/EDTA solution after confluence.

To evaluate the RK-13 culture conditions appropriate for rubella virus infection, cells ($1.3\sim1.5\times10^4$ per well) were plated on the 96-well cell culture plate with 0.2 mL basal MEM containing 0.5, 1, and 2% FBS. The cultures were incubated at 30, 33, and 35˚C (the temperature point of RA27/3 strain cold adaptation) for at least 14 days. The cells were then harvested with trypsin/EDTA and re-suspended in 10 mL of ISOTONE II solution. The cell numbers were determined by a Z1 Coulter Cell Counter (Beckman Coulter, Inc., FL).

III. *Rubella Potency Test and VSV Interference Test*

The potency test of live rubella virus vaccine followed the methods for potency testing of vaccines used in the World Health Organization's (WHO) expanded program on immunization[17]. It was conducted by the *in vitro* CPE microtitration method. Serial dilutions of vaccine samples were inoculated in rows of 10 wells of 96-well cell culture plates, together with 0.1 mL of trypsinized RK-13 cell suspension (1.5×10^4 cells per well). Then the plates were placed in humidified CO_2 incubators at different temperature setting based on the RA27/3 strain cold adaptation, and the cultures were incubated for 14 days. From Day 4, the CPE number in each dilution was read and counted daily. At day 7, the media of the RK-13 cultures were replaced with 0.2 mL of basal MEM containing 2% FBS per well. At day 10, the media were discarded and the cultures were inoculated with 100 to 300 cell culture infectious dose 50% ($CCID_{50}$) VSV per well of VSV. The cultures were further incubated at 33˚C and examined microscopically about 24 hrs later. Those wells where sufficient multiplication of rubella virus had taken place were protected against the growth and cytopathic activity of VSV. At the end of the testing period, the $CCID_{50}$ per human dose was acquired from the Reed and Muench method[18].

IV. *Validation Analysis of Rubella Potency Test*

In accordance with the validation analysis method of the International Conference on Harmonization (ICH) guidelines[19, 20], all the potency data underwent validation analysis to evaluate the accuracy and precision of rubella potency

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

test under different conditions. The SPSS statistic software (SPSS Inc., Chicago, IL, USA) was used to proceed validation and evaluation.

RESULTS AND DISCUSSION

In order to obtain a stable culture system for RK-13 cells to be infected with rubella virus, we examined several compositions of different serum concentrations and incubation temperatures. We found that RK-13 cell monolayer cultured in 96-well culture plate with MEM containing 2%FBS at 35˚C acquired the morphology of cell over-growth after 5 to 7-day cultivation (Figure 1B). The morphology of RK-13 over-growth was very similar to that of cells infected with rubella virus (Figure 1C). The results showed the lower culture temperature we selected, the longer the RK-13 monolayer could maintain (data not shown). The RK-13 cells had abnormal morphology included large plasma vesicles and

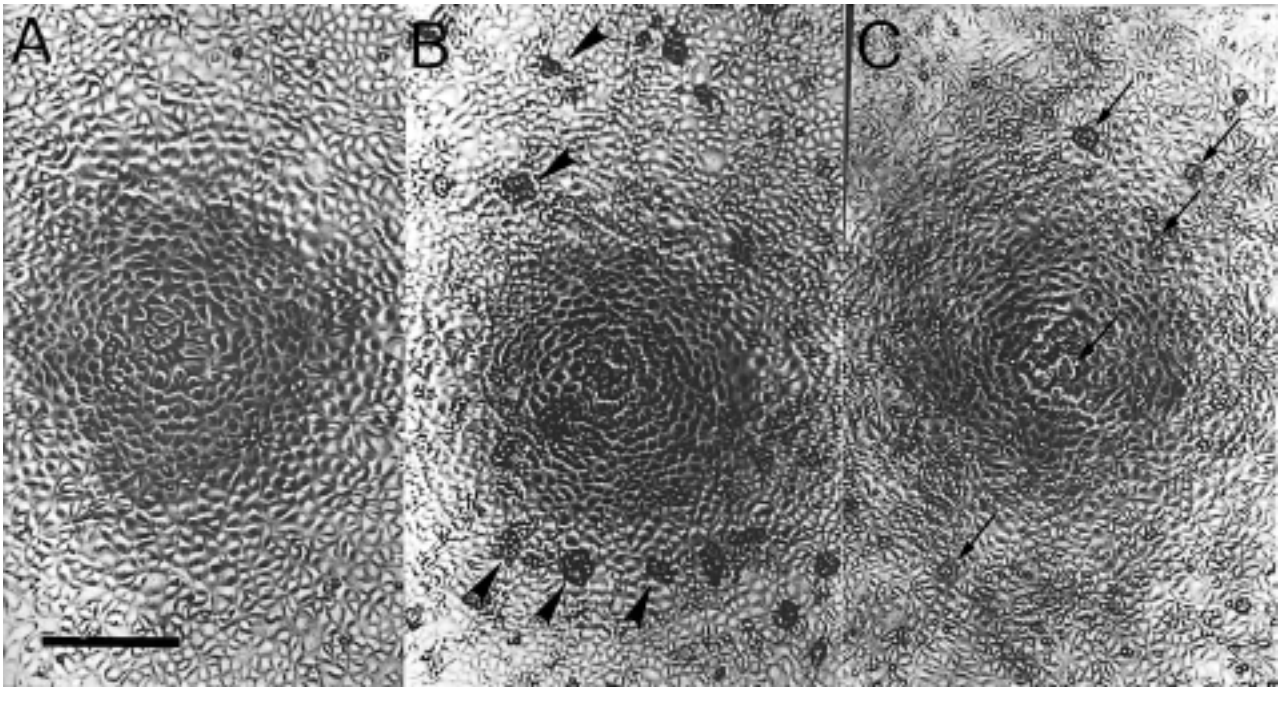

Figure 1. Phase contrast microscopic view of RK-13 monolayer infected with and without rubella virus. RK-13 cells were cultured in medium containing 2% FBS for 14 days at 33˚C with (C) and without rubella infection (A). Cell-overgrowth was appeared on RK-13 cells cultured in 2% FBS/MEM for 7 days at 35˚C (B). Arrow, morphology of rubella viral CPE; arrowhead, morphology of cell-overgrowth. Bar = 40 μm.

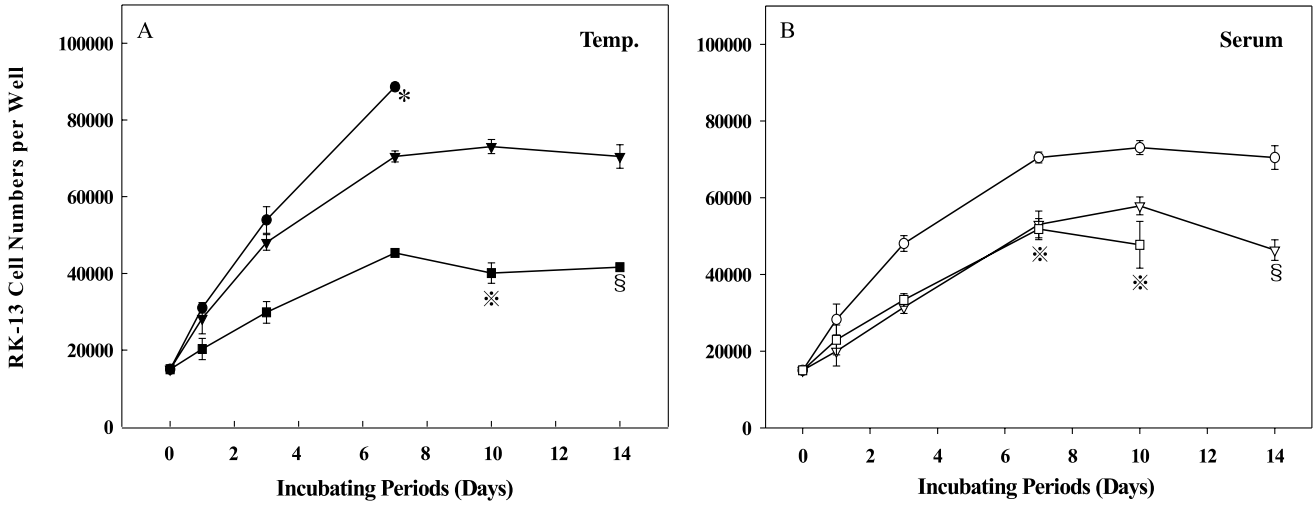

Figure 2. RK-13 cell growth response to different culture conditions. Cells were initially plated at 1.3×10^4 per well. Cells were cultured with MEM containing 2%FBS at 30, 33 and 35˚C for 14 days (A), or they were supplied with MEM containing 0.5, 1.0 and 2% FBS at 33 ℃ (B) for 14 days. Cell number was determined on Day 1, 3, 7, 10 and 14. Data are mean ± SD of triplicated determinations. ●, 35˚C and 2% FBS; ▼, 33˚C and 2% FBS; ■, 30˚C and 2% FBS; ○, 33˚C and 2% FBS; ▽, 33˚C and 1.5% FBS; □, 33˚C and 0.5% FBS. *, cell over-growth; ※, morphologic abnormality; §, cell death.

elongated pseudopodia (data not shown) while they were cultured at 30˚C for 10 Days and were not even able to form a confluent monolayer until the end of tests (Figure 2A). In addition, the lower serum starvations than 2%FBS also led to rounded sharp and dying at 33˚C incubation for 10 days (Figure 3B). However, the initial plating numbers of RK-13 cells also influenced the monolayer stability besides serum concentrations and culture temperatures. We tested several plating numbers and found not more than 13,000 cells per well was the suitable plating number for 14-day culture (data not shown). We suggest that RK-13 cells keep their normal morphology and physiological expression for testing periods only under the conditions of 13,000 cells per well, 2% FBS/MEM and 33˚C incubation.

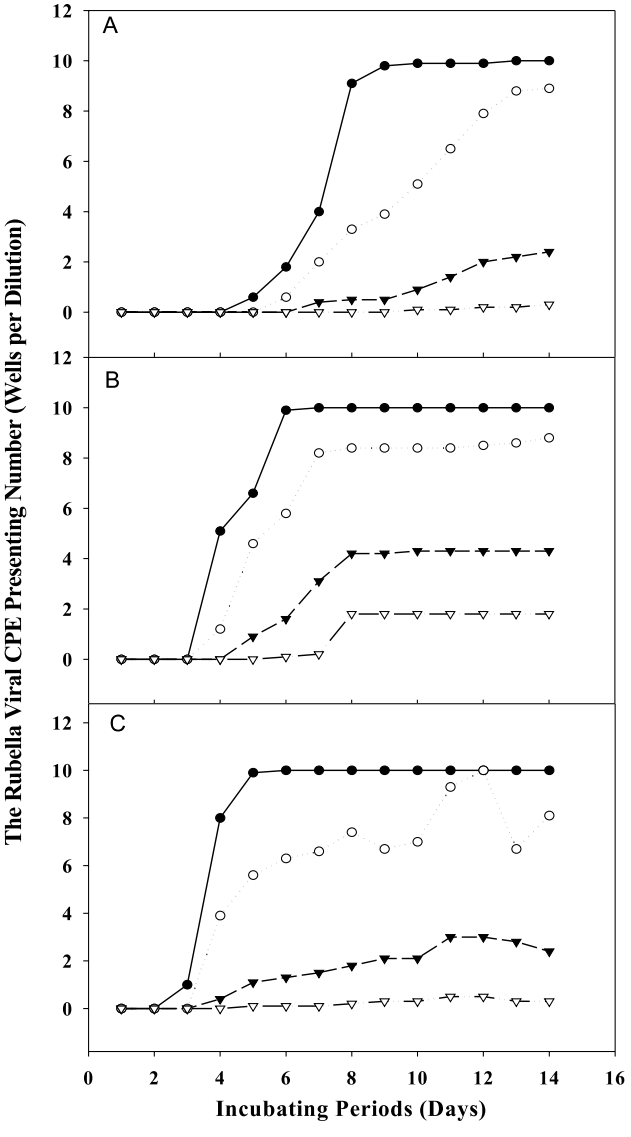

Figure 3. Rubella viral CPE presentation on 10-fold serial dilutions of rubella vaccine. Rubella vaccine sample was proceeding as 10-fold serial dilutions with 2%FBS/MEM. Each dilution of rubella inoculums was added into a 96-well plate of RK-13 monolayer by 0.1 mL per well with 10 duplicates. The cells were then incubated at 30 (A), 33 (B) and 35 (C)˚C for 14 days. Viral CPE was identified by phase-contrast microscopic examination. The number of CPE per well appeared in each dilution was recorded during Day 1 to Day 14. Data are mean of total determinations. ●, 10^{-1} dilution; ○, 10^{-2} dilution; ▼, 10^{-3} dilution; ▽, 10^{-4} dilution.

To evaluate the effects of RA27/3 strain virus infection under different inoculating/incubating temperatures, we conducted a series of *in vitro* CPE potency tests to compare the speed of CPE presentation and data stability among the 30, 33 and 35˚C incubating conditions. The results showed the rubella viral CPE of each diluted treatment appeared rapidly and reached a maximum on Day 8 under 33˚C incubation (Figure 3B). However, the CPE number appeared more slowly and only reached a maximum on Day 12 while the inoculum was incubated at 30˚C (Figure 3A). Although the numbers of viral CPE appeared most rapidly under the 35˚C incubating condition at 10^{-1} x dilution, the viral CPEs initially appeared more slowly and variably at 10^{-2} x, 10^{-3} x and 10^{-4} x dilutions (Figure 3C). This finding was similar to the testing results of RK-13 cell culture conditions, that was, it also supported that the condition of 33˚C and 2% FBS/MEM was the most suitable for rubella viral CPE identification.

To validate the results from the potency tests under different incubating temperatures, we analyzed the experimental data with several statistical methods. According to the ICH analytical procedure guideline[20], table two shows the experimental design for testing method validation of rubella vaccine potency. In this analytic matrix, the coefficient of variation (C.V., also called relative standard deviation, RSD) was used to estimate the repeatability for the triplicate of each potency test. In addition, we used one-way analysis of variation (one-way ANOVA) and Fisher's protected least significant difference (FPLSD) multiple comparison analysis to validate the accuracy and intermediate precision of total tests among all incubating conditions. In our results of validating analysis, the C.V. values of all potency tests were valid and ranged from 0 to 11.54%. The C.V. values from the tests under the 33˚C incubation were lower than ones from other two conditions (from 0.45 to 7.32% and from 1.72 to 11.54%) and ranged from 0 to 5.51% (Table 3). These results indicate that the 33˚C incubating condition provided the highest repeatability in each of the triplicate tests. Table four shows the results of FPLSD multiple comparison analysis among the 10-times tests. There was no significant difference among the average rubella potency under the 33˚C incubation. The difference among the tests was statistically significant ($p < 0.05$) under either the 30˚C or the 35˚C incubation. In addition, the results of one-way ANOVA also showed that there was no significant difference in the variance among 10-times potency tests ($p = 0.205$) in 33˚C incubating conditions (Table 5). However, the difference of variance among these tests was statistically significant under either the 30 or the 35˚C incubation. The CPE method of rubella potency test only conducted under the conditions of the 33˚C incubation and 2% serum concentration could hold enough sensitivity, accuracy and intermediate precision.

Because RK-13 monolayers infected with rubella virus were protected against the infection of VSV, technicians should avoid making a mistake about recognizing the real

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

Table 2. Experimental design for the evaluation on the *in vitro* CPE assay system of live rubella virus vaccine

Incubating Conditions	*In vitro* CPE Assay for Rubella Vaccine			
	Test 1	Test 2	----------	Test N
FBS: 2% Temp: 30˚C	Repeat 11 Repeat 12 Repeat 13 Mean 1 (n=3) SD C. V. *(Repeatability)*	Repeat 21 Repeat 22 Repeat 23 Mean 2 (n=3) SD C. V. (*Repeatability*)	---------- ---------- ---------- ---------- ---------- ----------	Repeat N1 Repeat N2 Repeat N3 Mean N (n=3) SD C. V. *(Repeatability)*
	FPLSD, N=X (Accuracy)			
	ANOVA (Intermediate Precision)			
FBS: 2% Temp: 33˚C	Mean 1 (n=3) SD C. V. *(Repeatability)*	Mean 2 (n=3) SD C. V. *(Repeatability)*	---------- ---------- ----------	Mean N (n=3) SD C. V. *(Repeatability)*
	FPLSD, N=X (Accuracy)			
	ANOVA (Intermediate Precision)			
FBS: 2% Temp: 35˚C	Mean 1 (n=3) SD C. V. *(Repeatability)*	Mean 2 (n=3) SD C. V. *(Repeatability)*	---------- ---------- ----------	Mean N (n=3) SD C. V. *(Repeatability)*
	FPLSD, N=X (Accuracy)			
	ANOVA (Intermediate Precision)			

Table 3. The repeatability evaluation for the *in vitro* CPE assay system of live rubella virus vaccine

Incubating Conditions	Test	Rubella Vaccine Potency ($Log_{10}CCID_{50}$/dose)									
		1	2	3	4	5	6	7	8	9	10
FBS: 2% Temp: 30˚C	Mean	*2.953*	*3.169*	*3.379*	*3.375*	*3.347*	*3.752*	*2.993*	*3.146*	*3.333*	*3.479*
	SD	*0.216*	*0.171*	*0.060*	*0.107*	*0.160*	*0.168*	*0.112*	*0.107*	*0.015*	*0.196*
	C. V.	*7.32%*	*5.41%*	*1.76%*	*3.16%*	*4.78%*	*4.49%*	*3.74%*	*3.40%*	*0.45%*	*5.63%*
FBS: 2% Temp: 33˚C	Mean	*3.476*	*3.227*	*3.262*	*3.337*	*3.671*	*3.337*	*3.186*	*3.217*	*3.350*	*3.320*
	SD	*0.056*	*0.028*	*0.088*	*0.174*	*0.048*	*0.174*	*0.122*	*0.117*	*0.000*	*0.109*
	C. V.	*1.63%*	*0.87%*	*2.71%*	*5.20%*	*1.31%*	*5.20%*	*3.82%*	*5.51%*	*0.00%*	*3.30%*
FBS: 2% Temp: 35˚C	Mean	*2.870*	*2.819*	*2.523*	*2.967*	*3.354*	*3.477*	*2.459*	*2.692*	*3.308*	*3.459*
	SD	*0.280*	*0.301*	*0.160*	*0.282*	*0.102*	*0.060*	*0.114*	*0.311*	*0.313*	*0.220*
	C. V.	*9.77%*	*10.67%*	*6.34%*	*9.50%*	*3.03%*	*1.72%*	*4.62%*	*11.54%*	*9.47%*	*6.37%*

Table 4. The results of FPLSD multiple comparison analysis among the different incubated temperature

Incubation Temperature		Rubella Vaccine Potency ($Log_{10}CCID_{50}$/dose)									
30˚C	Ranked Group	6	10	3	4	5	9	2	8	7	1
	Mean	*3.752*	*3.479*	*3.379*	*3.375*	*3.347*	*3.333*	*3.169*	*3.146*	*2.993*	*2.953*
33˚C	Ranked Group	5	1	9	4	6	10	3	2	8	7
	Mean	*3.671*	*3.476*	*3.350*	*3.337*	*3.337*	*3.320*	*3.262*	*3.227*	*3.217*	*3.186*
35˚C	Ranked Group	6	10	5	9	4	1	2	8	3	7
	Mean	*3.477*	*3.459*	*3.354*	*3.308*	*2.967*	*2.870*	*2.819*	*2.692*	*2.523*	*2.459*

Means underlined by the same line were not significantly different ($\alpha = 0.05$). The average potency from the 30˚C incubation among 10 tests were ranked and grouped into 4 groups. The average potency from the 35˚C incubation among 10 tests were also ranked and grouped into 3 groups. However, only the average potency from the 33˚C incubation were able to be pooled together. The results of 10 tests were homogeneous, that was, the 33˚C incubating condition could provide highly testing accuracy.

Table 5. The one-way ANOVA table of the potency test validation for live rubella virus vaccines

		Sum of Squares	df	Mean Square	F value	P value
Temp 30˚C	Between Tests	1.624	9	0.180	9.042	0.0000137[a]
	Within Tests	0.439	22	0.020		
	Total	2.062	31			
Temp 33˚C	Between Tests	0.638	9	0.071	1.151	0.205
	Within Tests	1.031	22	0.047		
	Total	1.669	31			
Temp 35˚C	Between Tests	4.322	9	0.177	8.201	0.0000296[a]
	Within Tests	1.018	22	0.022		
	Total	5.340	31			

[a] The rubella potency difference among 10 tests was statistically significant ($\alpha = 0.01$).

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

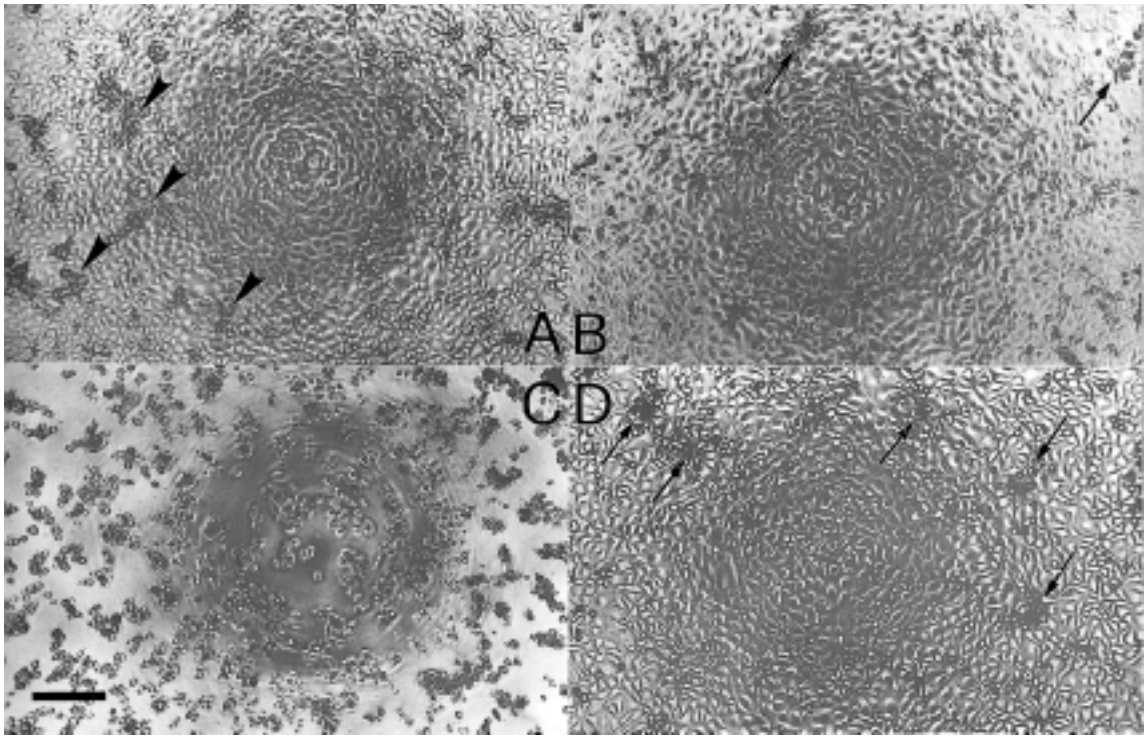

Figure 4. Phase contrast microscopic view of RK-13 monolayer infected with rubella virus and VSV. RK-13 cells cultured at 35˚C and formed the overgrowth morphology on Day 7 (A). These cells were infected with VSV on Day 12 and formed cytopathic morphology after 24 hrs later (C). Rubella virus infected RK-13 cells were co-infected with (D) and without VSV (B) in 2% FBS/MEM at 35˚C for 12~14 days. Arrow, morphology of rubella viral CPE; arrowhead, morphology of cell-overgrowth. Bar = 40 μm.

Table 6. The paired T-test analysis of vaccine potency resulted from rubella virus infectivity and VSV interference infection

Incubating Temperature	30 ℃		33 ℃		35 ℃	
VSV	−	+	−	+	−	+
Mean	3.2786	3.3202	3.3730	3.3868	2.9667	3.0502
Variance	0.0665	0.0595	0.0490	0.0460	0.1723	0.1825
dF	31		31		31	
T value	-3.3146		-1.3945		-3.1917	
P value	0.0023[a]		0.1731		0.0032[a]	

[a] The difference between the potency results from CPE testing method and VSV interference testing method was statistically significant ($\alpha = 0.01$).

rubella viral CPE and cell over-growth (Figure 4). Therefore, we used the paired T-test to compare the difference between the CPE results from microscopic examination under our suggested conditions with and without the VSV interference test. Table six shows there is no significant difference between the potency results acquired from our suggested testing condition and the VSV interference test. The differences between the CPE method and the VSV interference test were statistically significant ($p < 0.01$) under other culture conditions. Therefore, we suggest that the 2% of serum concentration and 33˚C incubating temperature could raise the sensitivity, repeatability and accuracy of CPE method for potency test of rubella vaccine. It also maintained the experimental precision similar to the results acquired from the VSV interference test. Furthermore, the optimal testing conditions we provided for determination of rubella vaccine potency enhanced the efficiency and precision, and these conditions also reduced the infectious risk of VSV as well as could promote the biosafety of laboratories. Although the repeatability, accuracy, and intermediate precision are essential testing method performance characteristics, and successful assay validation provides the data to assess these validated parameters[20, 21], assays for the live virus vaccine potency (virus content) can result in highly variable results due to the biological nature of the testing system. Furthermore, with the traditional *in vitro* CPE assay for potency determination of live rubella virus vaccine it is difficult to reduce assay bias and maintain testing repeatability, and easily leads to false positive or false negative potency results. Usually, variations come from cells, viruses, and culture conditions which can all significantly impact the results[7]. However, we have the responsibility to improve our testing accuracy, precision, rapidity and sensitivity for vaccine surveillance and lot release on the basis of our national authority.

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

CONCLUTIONS

In this study, we evaluated the CPE method for rubella potency test through statistical analysis. We found that serum concentration of cell culture and incubating temperature were the key factors influenced testing quality. Our results also suggested that the CPE potency test of live rubella virus vaccine could obtain the most accurate and precise results under the conditions of plating number of 13,000 cells/well, 2% of serum concentration, and 33˚C of incubating temperature.

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德國麻疹活病毒疫苗效價試驗方法之確效評估

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摘　要

一般而言，德國麻疹活病毒疫苗是利用病毒接種RK-13細胞產生離體細胞病變（cytopathic effect、CPE）的方法來測定其疫苗效價。然而，利用此法進行效價試驗時，透過顯微鏡來判讀德國麻疹病毒在RK-13細胞上產生的CPE並不容易。因此，對於政府相關單位及疫苗製造廠的檢驗實驗室而言，為確保德國麻疹活病毒疫苗的品質，發展精確、快速且有效的效價試驗方法是非常重要的。在本研究中，我們以不同的細胞培養條件來評估14天培養期中可使RK-13細胞維持正常形態的環境，並對在不同病毒接種環境下獲致之德國麻疹活病毒疫苗效價試驗CPE結果進行確效分析，以期找出最佳之德國麻疹疫苗效價試驗的方法。試驗結果顯示，當RK-13細胞培養以含2%濃度胎牛血清之培養液及33℃溫度環境中培養，可在96孔細胞培養盤中維持14天的正常形態與細胞生理，此結果提供德國麻疹疫苗進行CPE效價試驗時穩定的細胞基質環境。此外，我們發現33℃的病毒接種培養溫度在配合前述細胞培養條件之下，可使德國麻疹疫苗效價試驗的CPE呈時間自10至14天提前至7至8天，且易於鏡檢判讀，而在經由FPLSD多重比較、單因子變方分析及pair-T檢定等統計方法進行確效分析後發現，唯有在33℃的病毒接種培養環境下所獲得的效價結果，才能達到最佳的再現性（repeatability）、精密性（accuracy）與組間精確度（intermediate precision）。

關鍵詞：德國麻疹活病毒疫苗，細胞病變，確效分析