VIRUS CONTENT TEST OF FROZEN MAREK's DISEASE (MD) VACCINE USING SEVERAL DILUENTS

UJI KANDUNGAN VIRUS VAKSIN MAREK'S SEDIAAN BEKU MENGGUNAKAN BERBAGAI PELARUT

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ABSTRAK

Studi pengujian kandungan virus dari vaksin Marek's Disease (MD) bentuk sedian beku (frozen) telah dilakukan di Balai Besar Pengujian Mutu dan Sertifikasi Obat Hewan (BBPMSOH) Gunungsindur, Bogor dengan menggunakan berbagai pelarut yang berbeda komposisinya. Studi ini bertujuan untuk mengetahui kandungan virus vaksin MD sediaan beku (frozen) terhadap berbagai macam jenis pelarut. Beberapa perlakuan dari pemakai dilapangan menunjukkan bahwa kandungan virus tidak dipengaruhi dengan jenis pelarut yang digunakan. Lima jenis pelarut vaksin MD telah digunakan pada studi ini. Setelah vaksin MD dilarutkan dengan lima jenis pelarut berbeda, larutan yang telah ditentukan konsentrasinya dari masing-masing jenis pelarut diinokulasikan secara terpisah pada biakan jaringan selapis (monolayer) *Chicken Embryio Fibroblast (CEF)*. Inokulasi dilakukan pada menit ke 0, 10, 20, 30 dan 40 menit setelah dilarutkan. Hasil menunjukkan bahwa dengan menggunakan pelarut no 2, 3 dan 5 memberikan hasil kandungan virus yang stabil dengan perbedaan waktu inokulasi. Sedangkan dengan menggunakan pelarut 4, hasil kandungan virus MD paling tinggi dibandingkan dengan 4 pelarut lainnya. Secara keseluruhan titer virus dari vaksin beku MD semuanya memenuhi syarat (≥100 PFU) dengan menggunakan kelima macam pelarut tersebut.

Kata kunci : Vaksin *Marek's Disease*

ABSTRACT

Study of the virus content of the frozen type Marek's Disease (MD) vaccine using several kinds of diluents was carried out at National Veterinary Drug Assay laboratory (NVDAL). This test has carried out to know the virus content in frozen MD vaccine after several treatments of vaccine diluents. Several treatments from users in the field showed that the virus content of frozen MD vaccine was not influenced by manipulating of several diluents.Five diluents were used in this study. Diluted vaccines were inoculated separately onto chicken embryo fibroblast (CEF) monolayer for 0, 20, 30 and 40 minutes after diluting. The results showed that virus content of MD vaccine were stable in diluents 2, 3 and 5 in different time inoculation. Diluent-4 gave the highest virus content of frozen MD vaccine compared with 4 other diluents. However all of the virus titre of MD frozen vaccine are satisfied (more than 1000 PFU) after diluted in five kind diluents.

Key word : Marek's Disease (MD) vaccine

INTRODUCTION

Three strains of MD vaccines are circulated in the world. There are CVI / Rispens as strain I, SB-1 as strain 2 and HVT as strain-3., where those strains are provided to be two kinds of MD vaccines (freeze dried type and frozen type), available in Indonesian market, Both of them are used to immunize day old chicks (DOC). Especially for frozen Marek's Disease (MD) vaccine is usually used for immunizing DOC by diluted it using the original diluents itself and directly injected them into their neck as sub-cutaneously route to prevent the vaccine quality.

However at the field farms, some users has not applied diluted MD vaccines directly, but keep the diluting MD vaccine longer or take more time before it is used. Moreover many users make their formulas as MD vaccine diluents as their secret diluents formula.

To release MD vaccine assay in NVDAL, one of the minimum requirement to be passed is virus content, beside other requirements like general tests (sterility and purity) also special tests (safety and potency).

Plaque Forming Unit (PFU) as a titre value of virus content of MD vaccine must be more than 1000 PFU for frozen type and at least 1500 PFU for freeze dried type as stipulated under Indonesian and OIE minimum requirements. Especially for virus content, the MD vaccine must be diluted by maintenance minimum eagle medium or diluent itself and directly inoculate onto sensitive tissue culture. Since many users in the field manipulate the reconstitute of MD vaccine before it is applied in DOC, made author wants to know how about the titre of MD vaccine after manipulating with their secret formula as the users commonly done in the field.

MATERIALS AND METHODS

Chicken Embryo Fibroblast (CEF) monolayer was prepared under (Lestari, 1998) and Soedijar, *et al*, 2004 methods. CEF was provided using 9 days old specific pathogen free eggs.

Five flacons of frozen type MD vaccine were used and reconstituted in standard diluents as our routine diluents and also the vaccine diluents itself.

Diluents used in the test were *Diluent 1*, Diluent 1 is a NVDAL standard diluents contained minimum eagle medium 85%; trypto phosphate broth 10%; calf serum 1%; fungizone 1%; antibiotic 1%, $NaHCO_3$ 1%; L-glutamin 1%. *Diluent 2,* Four grams of antibiotic A (contents has no description) was diluted into 80 ml distillates water (DW_2). Two ml of this solution was added into 198 ml of vaccine diluent. *Diluent 3.* Four grams antibiotic A was diluted into 80 ml of B code solution. Two ml of this solution was added into 198 ml of vaccine diluents. *Diluent 4,* Four grams antibiotic A was diluted into 80 ml of B code solution (contents has no description). A volume of 2.5 ml of this solution was mixed with 197.5 ml of vaccine diluents. *Diluent 5*, A volume of 2.5 ml of antibiotic Z solution (contents has no description) from field originally was mixed with 197.5 ml of vaccine diluents.

The comprehensive of MD vaccines dilutions have carried out as our routine assay for the first reconstitute and were followed under field users manipulating method such as the follow procedures: One flacon of MD vaccine was diluted with 200 ml

diluents-1. A volume of 0.5 ml of this diluted vaccine was added into 9.5 ml diluents-1. Further, 3 ml of this dilution was mixed with 3 ml diluents-1 to be 40 times dilutions. Then 0.2 ml of the 40 times dilution was inoculated onto each of 4 (four) plastic petri-dishes which contained CEF monolayer and incubated at 37 °C, 5% CO_2 incubator for 1 hour. Four ml of maintenance medium was added and more incubated for 4-5 days. Plaque Forming Unit (PFU) was counted at the last incubation period. One flacon of MD vaccine was diluted with 200 ml diluents-2. And then 0.5 ml of this diluted vaccine was added into 9.5 ml diluents-1. From this mixture, 3 ml of sub- diluted vaccine was mixed with 3 ml diluents-1. Then 0.2 ml of the last dilution vaccine (40 X dilutions) was inoculated onto each of 4 (four) plastic Petri-dishes contained CEF monolayer after minutes of 20, 30 and 40 respectively and incubated at 37 °C, 5% CO_2 incubator for 1 hour. Then 4 ml of maintenance medium was added and kept for 4-5 days more. Plaque Forming Unit (PFU) then counted to give the titre of MD vaccine content. One flacon of MD vaccine was diluted with 200 ml diluents-3. A volume of 0.5 ml of diluted vaccine was added into 9.5 ml diluents-1. Further, 3 ml of sub-diluted vaccine was mixed with 3 ml diluents-1. Then 0.2 ml of the last dilution vaccine (40 X dilutions) was inoculated onto each 4 (four) plastic Petri-dishes contained CEF monolayer after minutes of 20, 30 and 40 respectively and incubated at 37 °C, 5% CO_2 incubator for 1 hour. Then 4 ml of maintenance medium was added and more incubated for 4-5 days. Plaque Forming Unit (PFU) was counted at the last incubation period. One flacon of MD vaccine was dissolved into 200 ml diluents-4. A volume of 0.5 ml of this diluted vaccine was added into 9.5 ml diluents-1. Further, 3 ml of sub-diluted vaccine was mixed with 3 ml diluents-1. Then 0.2 ml of the last dilution vaccine (40 X dilutions) was inoculated onto each 4 (four) plastic Petri-dishes contained CEF monolayer after 20 min, 30 min and 40 min respectively and incubated at 37 °C, 5% CO_2 incubator for 1 hour. Four ml of maintenance medium was added and more incubated for 4-5 days. Plaque Forming Unit (PFU) then counted to give the titre of MD vaccine content. One flacon of MD vaccine was diluted with 200 ml diluents-5. Further 0.5 ml of this diluted vaccine was added into 9.5 ml diluents-1. Three ml of sub-diluted vaccine was mixed with 3 ml diluents-1. Then 0.2 ml of the last dilution vaccine (40 X dilutions) was inoculated onto each 4 (four) plastic Petri-dishes contained CEF monolayer after 20 min, 30 min and 40 min respectively and incubated at 37 °C, 5% CO_2 incubator for 1 hour. Then 4 ml of maintenance medium was added and re-incubated for 4-5 days. Plaque Forming Unit (PFU) occurred and PFU average was calculated to give the titre of MD vaccine content.

RESULT AND DISCUSSION

Standard diluents (diluents-1) was applied in our laboratory is a NVDAL method as representative using diluents-1 for 0 minute (directly inoculate after reconstitution vaccine). It was appeared that diluents-1 gave PFU (virus content) decrease as rhythm as increasing inoculation period. This is understood that the length keeping time make the particle survive virus decrease and gave the growth PFU in tissue culture also decrease as consequence. But when it was inoculated for 40 minutes after

diluting the vaccine gave greater virus content than other different inoculation period which is not expected as shown at table 1.

Table 1. Plaque Forming Units (PFU) result after inoculation at 0, 20, 30 and 40 minutes.

Diluent	Results (PFU)			
	0 minutes	20 minutes	30 minutes	40 minutes
1	1900	1584	1492	1624
2	1870	2160	2130	2110
3	2390	3180	2510	2300
4	4790	4210	5380	4730
5	2420	2610	2360	2300

Both of the diluents-3 and diluents-4 gave fluctuation virus titre in different period. These diluents-3 and diluents-4 also gave higher virus content than other diluents. Especially diluents-4 gave the highest virus content compare than other diluents (figure 1). It could not be described more detail since author also did not know the exact medium / diluent composition.

Experiment using diluents-4 gave the highest titre when it is compared with other diluents as it shown at figure 1. It might be asked to the users what was composition of their diluent.

Unpredictable was diluents-1 as our routine diluent gave the lowest titre amongst other diluent, although there was no unsatisfied titre under several manipulations for vaccine dilution as shown at figure 1.

Each of all diluents used diluent-1 as standard diluent before diluting into their respective diluents. For further experiment needs to know the virus titre result if they use only their diluents itself without mixing with standard NVDAL diluents.

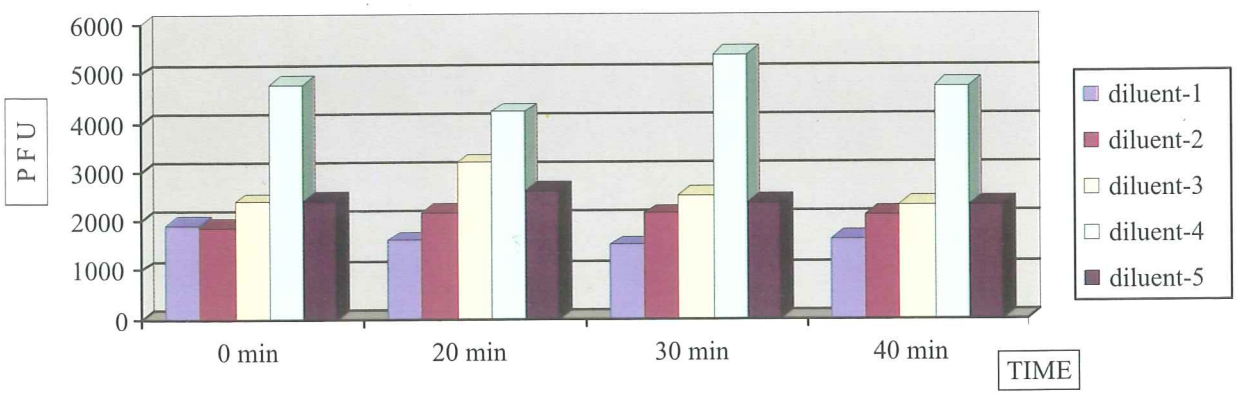

Figure 1. Hortograme of PFU of MD vaccine using several diluents after inoculations

Three diluents were diluents-1, diluents 2 and diluents 5 gave relative stable virus content (PFU) during different inoculation period as presented at figure 1.

All frozen MD vaccines assayed in this study were satisfied when diluted under several methods since they favour more than 1000 PFU (anonymous, 2007 and Payla, V, 1997).

Though diluents-4 gave the highest PFU, however all MD vaccine titre were satisfied since deliver more than 1000 PFU. To make sure the virus content as field users applied, further experiment using only their diluents without standard (NVDAL) diluents is needed to perform.

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