Summary Report of Activity

Student Exchange Program (MEXT PROJECT)
Collaboration Airlangga University (Indonesia) and Kobe University (Japan)

&

Exchange Program
Faculty of Health Science, Kobe University
Myodani Campus, Kobe, Hyogo Prefecture, Japan
2013

Report of Activity

Supervisor **: Prof. Hak Hotta**
Sensei : Masanori Kameoka, PhD
Name of Student : Baharuddin Badi, S.Si (equivalent B.Sc)

Introduced of Instruments and Medium which used for Cell Culture and Dengue Virus Experiment :

Purpose : For knew all instruments and understand how to operations that instruments by safely, during experiment and also knew the kind of medium in cell culture and Dengue Virus (DEN) experiment.

Results :

1. RPMI-1640 Medium from Sigma-Aldrich®:

Descriptions compositions are:
RPMI-1640 Medium (500ml) + FBS 10% + 1 % *Penicillin-Streptomycin*. This is the media composition for K562 cells culture.

2. Minimum Essential Media (MEM), the media powders is made by **Nissui Pharmaceutical** Co., Ltd. :

Descriptions compositions are:
Medium MEM 500ml + FBS 10% + 1% *L-Glutamine* + Adjust pH

Conclusions :

1. RPMI-1640 Medium from **Sigma-Aldrich**®: is very good to applied for cell culture, in this case is K562 cell (semi adherent type), because this media has compound macro and micro element such as: Inorganic salts, Amino acids, Vitamins, and Carbohydrate (D-glucose).
2. The MEM (Minimum Essential Media) Media from **Nissui Pharmaceutical Co., Ltd**. : is very good to applied for cell culture in this case is Vero cell (adherent type), because this media has compound macro and micro element such as: Sodium chloride, Amino acids, Vitamins, Carbohydrate (glucose) and Kanamycin.
3. All Instruments and Material such as medium before and during was working on cell culture must be sterile and condition must aseptic for prevent there are contaminations.

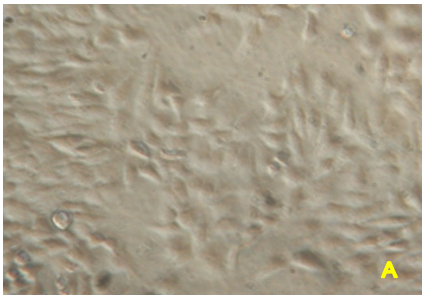
Cell Culture Experiment :

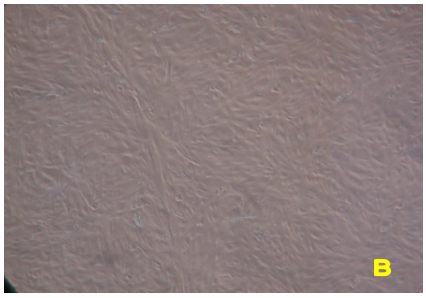
Purpose :

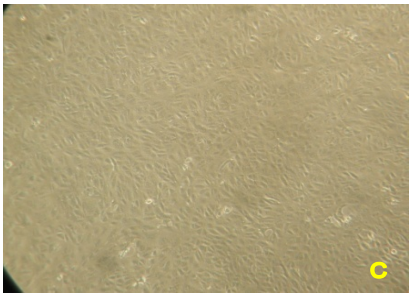
1. To know how to making media for K562 cell and vero cell.
2. The purpose for this session is to rejuvenate, growth up and increased the numbers of cells.

Results :

1. Pictures of Vero cells.



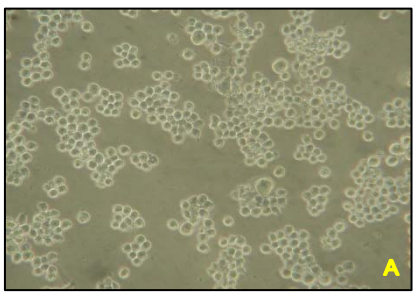




Descriptions:

A. Vero cell the age of 3 days with confluent 50%.
B. Vero cell the age of 5 days with confluent 70%.
C. Vero cell the age of 6 days with confluent 100%.

2. Pictures of K562 Cells.



Descriptions:

A. Vero cell the age of 2 days with confluent 40%.
B. Vero cell the age of 3 days with confluent 90%.
C. Vero cell the age of 5 days with confluent 100%.

Conclusions :

1. The Vero cell had we used is a cell line which isolated from kidney epithelial an African green monkeys by scientists. This cell is type adherent is caused very strong to attached on polystyrene Tissue Culture Flask Falcon®. So because of that we used *Trypsin EDTA* has sterilized with filter, for detached it. For culture of Vero cell we used MEM media with compound of main compositions is : MEM 500 ml + FBS 10% + L-Glutamine 1% + Adjust pH with $NaHCO_3$ (4-6 ml) and the result is good.

2. The K562 cell had we used is a cell line from erythroleukimia and this is a semi-adherent type it caused could not strong to attached on polystyrene Tissue Culture Flask Falcon® such as T75 or T25.

3. The growing rate of cell is caused of many factors such as condition of media. The compositions every elements in media must appropriate necessary of cell. After that the second important is conditions during incubations period, in this case pointing at CO_2 concentrations and the set of temperatures, if there is imbalance the growing rate of cell would become to lower and continue to die.

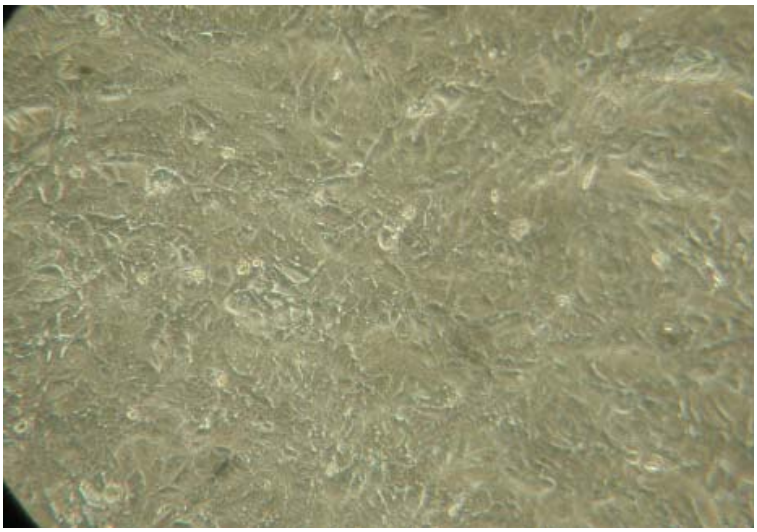
Dengue Virus Infections Experiment :

Purpose :

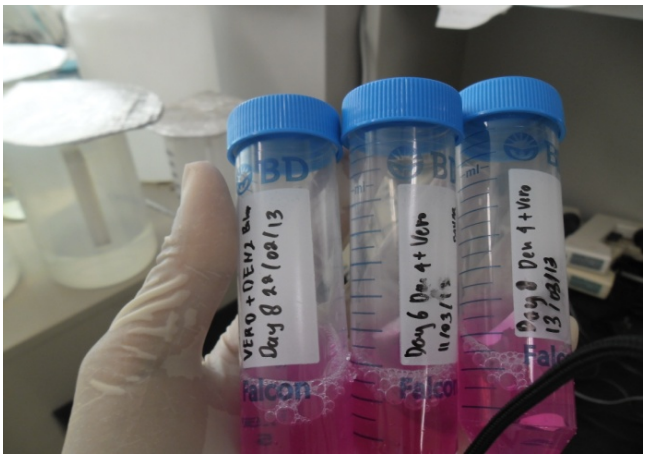
1. For understand about CPE (*Cytophatic Effect*) while Dengue Virus is infected to Vero cell.
2. For getting much more of Dengue Virus quantity.
3. The first activity for count the titer of virus obtained last time harvested process.

Result :

1. The Vero cell which experienced of CPE (*Cytophatic Effect*) because infected of Dengue Virus (DEN).



2. The solution which containing Dengue Virus from Harvesting process with an additional $NaHCO_3$ 7,5% for adjust pH.



Conclusions :

1. The CPE "*Cytophatic Effect*" we can see it on sixth or more days. The wrinkling that was Indication of CPE on the cells this is caused of membrane was being damaged and if this injury has severed can make a cell to die.
2. Harvesting process we done on fourth, sixth and eighth day. All solution from T75 which compound of Dengue Virus taken and put into 50 ml Falcon tube and centrifuged with used 3.000 rpm within 5 min. After that, added $NaHCO_3$ for adjust pH and save in freezer -80^0C.

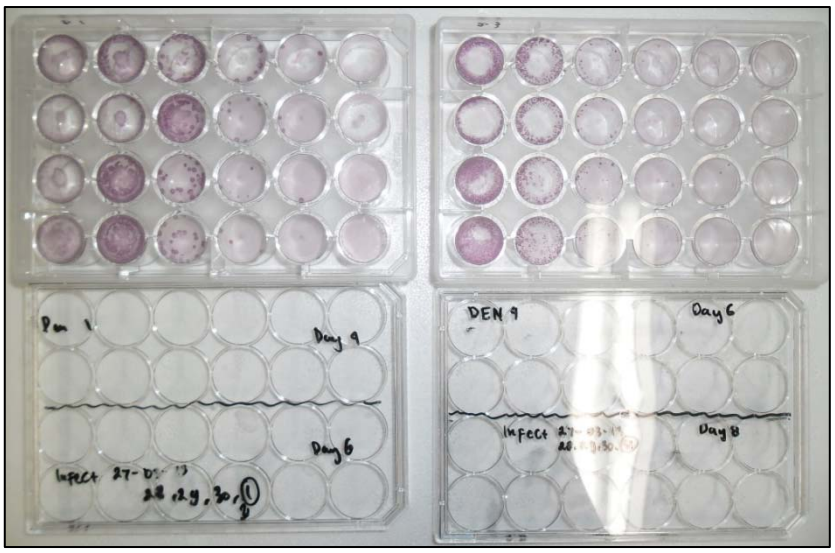
Immunostaining:

Purpose :

For knew a titer rate level in our sample so that we can make appropriate dilutions.

Result :

One of result staining process for Dengue Virus for making a titer.



Descriptions: The result of staining process on M24 plates by last time which incubation of Vero cell and infected with Dengue Virus (on pictures is showed DEN 1 day 4,6 and DEN 4 day 6 and 8).

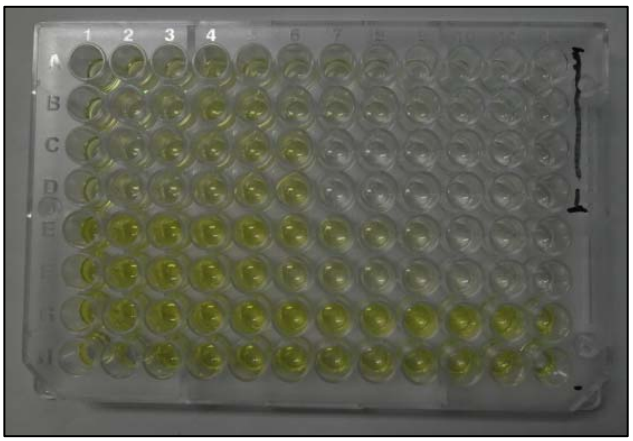
Conclusions :
Counting of virus titer. Calculations performed on the virus titer plate M24 (Falcon ®) with a volume of 1 ml @ well is, who previously performed multilevel dilution or dilution by using plate M96 (Falcon ®). This procedure is performed to determine the titer of virus stock owned. Of this process can also note that the size of the plaque that forms on any type of virus can be different where the image size is smaller plaque DEN 2 compared with DEN 1.

ElISA for Dengue Virus Experiment :
Purpose :

1. For checks there are or no Dengue Virus on samples.
2. For correction whether an antigen has appropriate to antibody.
3. To compares with dengue virus dilutions.

Result :

1. Result from ELISA process which event reactions antigens and antibodies.



Conclusions : For the ELISA test we used Maxi SORP plate 96 NUNC™ because the bottom of the layer (stationary phase) can strongly capture antibody. For antibody (capture) we used anti NGC dengue (DEN 2) This process is known as " coating antibody " after that we added antigen in this case is dengue virus DEN 2 NGC strain (New Guinea Strain) and primary antibody (primary detector) we added this is an universal antibody for dengue (4G2), (IgG) which is an "ascites fluid" or MAb from mice. The next stage, added a secondary antibody (anti-mouse IgG) alkaline phosphatase labeled substrate PNPP then and incubated, and read on a machine reader in experimented we used instrument from Bio-Rad.

2. Result from reader ELISA process.

23-03-2013 time: 19:01:08					Bio-Rad Laboratories							
Measurement Filter : 415 nm												
Reference Filter : 655 nm												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.044	0.035	0.032	0.039	0.027	0.018	0.021	0.013	0.010	0.006	0.002	0.004
B	0.053	0.045	0.047	0.053	0.046	0.025	0.019	0.017	0.012	0.009	0.002	0.001
C	0.065	0.057	0.059	0.058	0.055	0.048	0.023	0.014	0.015	0.005	0.003	0.007
D	0.051	0.049	0.046	0.049	0.047	0.044	0.015	0.011	0.007	0.009	0.002	0.000
E	0.540	0.247	0.192	0.093	0.079	0.044	0.031	0.016	0.011	0.001	0.002	0.005
F	0.674	0.283	0.197	0.107	0.066	0.028	0.022	0.016	0.017	0.010	0.006	0.006
G	0.508	0.501	0.524	0.809	0.574	0.647	0.056	0.054	0.104	0.102	0.186	0.270
H	0.477	0.410	0.738	0.736	0.643	0.676	0.064	0.065	0.109	0.118	0.229	0.301

Conclusions : With using Reader Instrument from Bio-Rad we can see there's decreased of quantity of Dengue Virus (DEN) which indicated low of OD (optical density) value near to zero "0" point when dilution upper 10^9 special for DEN 1 day 8 and DEN 2 day 8.

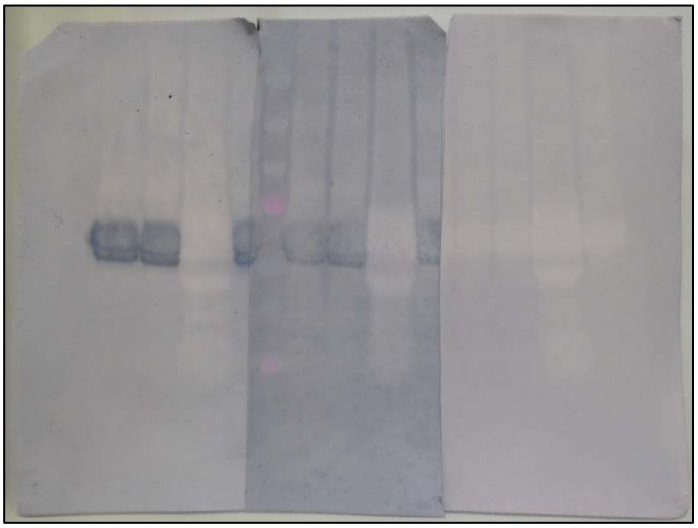
Western Blot Experiment :

Purpose :

Function of the Western Blot is to detect the presence or absence of proteins in a sample, where the protein was transferred to a special membrane which further stained in order to visualized.

Result :

1. Result from Western Blot process.



Conclusions :

1. Western blot procedure consists of sample preparation, gel electrophoresis, transfer from gel to membrane, and immune staining of the blot.
2. Principles used in the western blot is the principle of antigen-binding complex atibodi. Protein in NC we think of as antigen. Primary antibody is an antibody that can bind specifically to antigens in NC. To be able to see the bonding of the antigen-antibody complex then we give color to the antigen-antibody.
3. Of this process can be seen in the form of viral protein antigens in a sample which is determined by comparing the band positions obtained with Marker position beside him.

Thank You Very Much.

Report of Activity

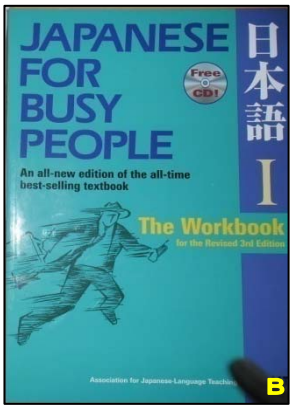
Sensei : Prof. Satoshi Terao
Assistant : Miyako Kiso (PhD. Student)
Nama Student : Baharuddin Badi, S.Si

Japanese Language and Culture
Purposes :

1. To Know and understand rules of Japanese Language.
2. To know and understand about Japanese Culture.

Result:

1. Japanese Language and Culture session class.



Descriptions:

A. While teaching and learning process in the classroom.
B. The main handbook which use during in Japanese Language class.

2. Trip to around of Kobe University in Rokko highland area.

Descriptions:

A. Meet and acquainted with Indonesian students which are studying at Kobe University.
B. The hospital in Rokko campus has functions to support the health every students and teachers during school at Kobe University and for realize that every year, every students must come to here for check up his/her conditions.

3. Excursion to Center of Kobe Museum for learn the lives of Japanese citizens from age to age.

4. While was meeting with Mr. Takayoshi Kuno M.D Ph.D. and team from The Center for Education of Global Leaders for ASEAN and Students Indonesian-Japan Exchange Project and some students from Medical Faculty of Kobe University.

Conlusions :

1. In Japanese Language there are three of characters which compose a sentences. The characters are used is Kanji, Hiragana and Katakana. Kanji characters is came from Chine and Hiragana is origin words from Japan but Katana characters use to determine a something of foreign.

2. The daily of Japanese people show a habit of disciplines and always focus to finish his/her jobs.
3. The Japan country has become a great country in global market because they always doing innovation to them products.
4. The government of Japan has prevented trap jam with uses electrical trains, and on the streets the government has implemented strict regulation for control of quantity of vehicle which will reduce pollutions on the air.
5. The country of Japan very susceptible for earthquake because the Japan island standing on top of the oceanic plate and continental plate which not too stabile, so every peoples which stay in Japan must know how to indication event the earthquake and know of the warning system.

Thank You Very Much.