Report

Regional Training on Vaccine matching for analysing the homology of field isolates/strains in relation to the in-use vaccine strain(s) of foot and mouth disease

29 October to 7 November 2012
Mukteswar, India

European Union

Contents **Page**

Acronyms and abbreviations 3

Summary 4

Background 6

Day wise activities (Day 1 to Day 10) 8

Conclusions and recommendations 16

Closing remarks 17

Annex 1 Agenda 18

Annex 2 List of Participants 22

Annex 3 Summary of Assessment of Regional Leading Diagnostic Laboratories 25

Acronyms and abbreviations

BEI	Binary Ethylenimine
BHK	Baby Hamster Kidney
BVS	Bovine Vaccinate Serum
CPE	Cytopathic Effects
DGR	Dangerous Goods Regulations
ECTAD	Emergency Center for Transboundary Animal Disease
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GLP	Good Laboratory Practice
HPAI	Highly Pathogenic Avian Influenza
HPEDs	Highly Pathogenic Emerging Diseases
IATA	International Air Traffic Association
LPB-ELISA	Liquid Phase Blocking ELISA
2D-MNT	Two Dimensional Micro Neutralisation Test
OD	Optical Density
OIE	World Organization for Animal Health (*Office International des Epizooties*)
PCP	Progressive Control Pathway
PD-FMD	Project Directorate on Foot and Mouth Disease
RLDL	Regional Leading Diagnostic Laboratory
RSU	Regional Support Unit
SAARC	South Asian Association for Regional Cooperation
SN	Serum Neutralisation
TCID	Tissue Culture Infective Dose

Summary

The Food and Agriculture Organization of United Nations (FAO) is implementing an European Union (EU) funded regional project (OSRO/RAS/901/EC) entitled "Regional Cooperation Programme on Highly Pathogenic and Emerging Diseases (HPED) in South Asia" under the umbrella of South Asian Association for Regional Cooperation (SAARC) at FAO Sub-regional ECTAD Unit in Kathmandu, Nepal. The overall objective of the project is to strengthen and empower SAARC nations in their ability to prevent, control and eradicate HPEDs, including HPAI, FMD etc. through improved veterinary and public health services and inter-sectoral collaboration on a regional basis.

The SAARC member counties have identified Project Directorate on Foot and Mouth Disease (PD-FMD), Mukteswar, India as the Regional Leading Diagnostic Laboratory (RLDL) for foot and mouth disease (FMD). The RLDLs are mandated to form, coordinate and lead the regional network of laboratories. The regional laboratory network will maintain uniform diagnostic standards, support training of laboratory scientists/technicians and backstop regional surveillance and epidemiological studies. The networking activities will be supported by the international OIE and FAO reference laboratories.

A number of laboratory activities have been implemented by the "Regional Cooperation Programme on Highly Pathogenic and Emerging Diseases (HPED) in South Asia" including trainings in diagnosis of FMD, need assessment of the laboratories, training on proficiency testing and setting up the FMD virus typing facilities in the national laboratories and epidemiology laboratory networking workshops.

Foot and mouth disease is endemic in SAARC region. All the three serotypes of O, A and Asia1 are prevalent with genetic and antigenic diversity and fall into gene pool 2 of FMD viruses. In such a situation assessment of appropriateness of in-use vaccine strains is very much essential. Antigenic differentiation/vaccine matching of field isolates is important for epidemiological studies of the disease and for selection of suitable vaccine strains. Vaccine matching is a crucial element in control of foot and mouth disease by vaccination. As a continuing step in the FMD control, a training programme on 'vaccine matching' for analysing FMD field isolates and vaccine strain/s was organised at the SAARC Regional Leading Diagnostic Laboratory at PD-FMD, Mukteswar, India.

The topics covered in the training included lectures and laboratory procedures including the design and operation of vaccine matching schemes, virus titre calculation, and determination of relationship (r1 values) with in-use vaccine strains. The training programme covered the requirements of the International Standards for vaccine matching tests.

The training was attended by 15 participants from the SAARC countries including Bangladesh, Bhutan, India, Nepal, Pakistan and Sri Lanka with technical support from FAO Regional Support Unit/Sub regional ECTAD for SAARC countries, Kathmandu, Nepal. During the nine days training programme, the participants were introduced with current status of FMD in Asia, including importance of vaccine matching. A lecture on collection, preservation and transportation of FMD samples including hands-on demonstration on processing of FMD clinical materials was also made. Demonstration made on sub-culturing of BHK-21 cells followed by infection of BHK-21 cells with clinical material and subsequently participants did on their own. The cytopathic effects (CPE) that occur after infection with FMD virus was demonstrated at different stages in comparison to the uninfected healthy cell monolayer. Harvesting of cell culture adapted viruses and their storage was demonstrated. Participants performed titration of FMD virus - by sandwich-ELISA to determine the optimum dilution of the reagents to be used (e.g. coating, tracing and antigen dilutions) in Liquid Phase Blocking ELISA (LPB-ELISA). The participants also performed the titration of FMD virus in cell culture to determine the tissue culture infective dose ($TCID_{50}$) of the virus. Subsequently the participants performed the vaccine matching by two-dimensional micro-neutralization (2D-MNT) test and liquid phase blocking ELISA (LPBE). The trainees were also assigned with the SN_{50} titration results to calculate SN_{50} titres and 'r' values to acclimatise with the calculation of the titres in 2D-MNT and LPBE and plot the regression curve analysis. Each subgroup was given different values and the group individually calculated the SN_{50} titres and r1 values. Presentation was also given on basic cell culture techniques and inactivation protocol of FMDV by using BEI.

Background

The Food and Agriculture Organization of United Nations (FAO) is implementing an European Union (EU) funded regional project (OSRO/RAS/901/EC) entitled "Regional Cooperation Programme on Highly Pathogenic and Emerging Diseases (HPED) in South Asia" under the umbrella of South Asian Association for Regional Cooperation (SAARC) at FAO Sub-regional ECTAD Unit/Regional Support Unit in Kathmandu, Nepal. The overall objective of the project is to strengthen and empower SAARC nations in their ability to prevent, control and eradicate HPED, including highly pathogenic avian influenza (HPAI), foot and mouth disease (FMD) and *peste des petits ruminants* (PPR) etc. through improved veterinary and public health services and inter-sectoral collaboration on a regional basis.

The SAARC member counties have identified Project Directorate on foot and mouth disease (PD-FMD), Mukteswar, India as Regional Leading Diagnostic Laboratory (RLDL) for FMD in the SAARC region. The mandate to the laboratory is to form, coordinate and lead regional network of FMD laboratories. The regional laboratory network will maintain uniform diagnostic standards, support training of laboratory scientists/technicians and backstop regional surveillance and epidemiological studies. The networking activities will be supported by the international OIE and FAO reference laboratories.

A number of laboratory activities have been implemented by the "Regional Cooperation Programme on Highly Pathogenic and Emerging Diseases (HPED) in South Asia" including trainings in diagnosis of FMD, need assessment of the laboratories, training on Proficiency testing for FMD and set up FMD virus typing facilities by the respective National FMD laboratories. Apart from these several workshops were organised.

In view of the on-going and upcoming PCP-FMD control programmes in the SAARC Member States, using vaccination, assessment of appropriateness of vaccine strains that are being used in the current vaccines is very much essential for successful control of the disease.

As a continuing step in the FMD control, a training programme on vaccine matching for analysing FMD field isolates and vaccine strain/s was organized at SAARC Regional Leading Diagnostic Laboratory at PD-FMD, Mukteswar, India.

The antigenic differentiation/vaccine matching of field isolates is important for epidemiological studies of the disease and for selection of suitable vaccine strains. This provides important clues for confirming the breaches in vaccinal immunity on account of appearance of new field strains as well as for deciding the suitability of vaccine strain(s) to be used in a particular situation for effective prophylactic and control measures for the disease.

The Regional Support Unit, based within the FAO's Sub-regional ECTAD Unit in Kathmandu, in collaboration with the RLDL on FMD organised a "Regional Training on vaccine matching for analyzing the homology of field isolates/strains in relation to the in-use vaccine strain(s) of foot and mouth disease" from 29 October to 7 November 2012 with support from the Government of India, Indian Council of Agricultural Research, SAARC and European Union.

Objectives of the training:

The main objective of this training was:

1. To train staff of National Laboratories of the member countries in conducting vaccine matching exercise by 2D-MNT and LPBE.

Expected outcomes:

i. The National Laboratories will develop capacity for FMD vaccine matching through selecting most appropriate vaccine strains to be used in a country
ii. Monitoring the appropriateness of in-use vaccine strains
iii. Provide important epidemiological information on the circulation of antigenically different field isolates and causes of vaccination failures

Day wise activities

Day-1

The training was opened by a welcome address by Dr Venkatasubbarao Mandava, Laboratory Coordinator, Regional Support Unit/Sub Regional ECTAD, FAO, Nepal and Dr B Pattnaik, Project Director, PD-FMD, Mukteswar. The participants were taken for visit to the Central FMD Laboratory, PD-FMD.

A presentation was made on FMD, current status in Asia, India and plans and preparedness in India by Dr A. Sanyal. He narrated that as per OIE and FAO, countries having FMD are more prone to food insecurity as a result of the impact of FMD at household level through reduced access to local, national and international markets and animal draught power for agriculture. He talked about the distribution of FMD virus serotypes, disease prevalence and proximity to FMD endemic neighbouring countries. It was evident that serotype O is the most prevalent serotype in all the countries in SAARC region. Dr J. K. Mohapatra, PD-FMD delivered a lecture about vaccine matching and its importance. He explained different methods of vaccine matching techniques. A lecture on collection, preservation and transportation of FMD samples was delivered by Dr S. Saravanan, PD-FMD. He also discussed about OIE manual regarding DGR (Dangerous good regulations) and IATA (International Air Traffic Association) for transporting infectious material.

The laboratory procedures started with a demonstration on the processing of FMD suspected tissue or clinical materials followed by hands on training on processing of the clinical material by groups of participants. This was followed by demonstration on preparation of media, use of appropriate glass/plastic wares, trypsin, equipments and preparation of other reagents needed for cell culture preparation. Demonstration was also made on sub-culturing of BHK-21 cells followed by infection of BHK-21 cells with clinical material processed by the groups.

Day-2 (30-10-12)

The day started with review of work of the previous day and interactive session with the trainers. The participants in groups went to cell culture laboratory for sub culturing of BHK-21 cells, preparation of virus inoculums, and inoculation of healthy cell monolayer with processed clinical samples. The cytopathic effects/changes (CPE) that occur after infection with FMD virus was demonstrated at different stages in comparison to the uninfected healthy

cell monolayer. Harvesting of cell culture adapted viruses and their storage was demonstrated.

In the evening session titration of FMD virus in cell culture was demonstrated. Participants were further divided into small groups of two and asked to perform titration of FMD virus - by sandwich-ELISA. All the participants were taught about all aspects of sandwich-ELISA including washing plates and recording of O.D. values using ELISA reader. Results were recorded by all groups and discussed with the trainers for accuracy.

The participants of Group 2 performed the virus titration by sandwich-ELISA to determine the optimal dilution of antigen to be used using one vaccine virus strain and four field viruses. Results obtained by different subgroups are summarised below:

Isolates	**Group 2A**	**Group2B**	**Group 2C**
V1	1+8	1+14	1+8
V2	Neat	Neat	Neat
V3	neat	Neat	Neat
V4	Neat	Neat	Neat
V5	>1+12	>1+12	>1+12

The results were identical across subgroups which showed reproducibility, robustness and ruggedness of the kit used for sandwich-ELISA. The group was also demonstrated virus titration in BHK-21 cells. The group performed virus titration independently using two virus isolates viz. IND40/00 and PD213/12. The plates were stained after 48 hours once the cell controls formed a complete monolayer while infected wells showed CPE in the plates on 1 November 2012 (4th day of the training). Initially the group was taught how to calculate the virus titre. Then all the subgroups calculated the titre independently.
The results are tabulated below:

Virus isolates	**2A**	**2B**	**2C**
A40/00	$10^{6.175}$	$10^{6.175}$	$10^{6.925}$
PD213/12	$10^{7.475}$	$10^{7.3}$	$10^{6.675}$

The titre of virus were expressed as $TCID_{50}$/ml

Day-3 (31-10-2012)

After reviewing the previous day’s work and discussion, Group 1 started the day by performing the LPBE test. The principle of the vaccine matching test (LPBE) was explained and demonstrated to the group. The group performed titration of the freeze dried inactivated virus antigen to determine the optimum dilution of the reagents to be used (e.g. coating,

tracing and antigen dilutions). In the evening session reagents for LPBE like coating buffer, coating of microtitre plates, two-fold dilutions of bovine vaccinate serum (BVS) were done by the participants. The diluted virus and BVS were mixed in a Perspex plate for overnight incubation at 4° C.

The principle of vaccine matching by two-dimensional micro-neutralization (2D-MNT) test was explained -. The test was performed and kept at 37° C for 48-72 hrs.

Result of serotype "O" LPBE

Group	Virus	Serum titre	r_1 Value
1a	INDR2/1975	354	
	Test Virus1	354	1
	Test Virus2	177	0.5
1b	INDR2/1975	445	
	Test Virus1	445	1
	Test Virus2	354	0.75
1c	INDR2/1975	707	
	Test Virus1	707	1
	Test Virus2	707	1
1d	INDR2/1975	707	
	Test Virus1	707	1
	Test Virus2	707	1

The Group 2 performed 2D-MNT in BHK-21 cells followed by hands on performance of 2D-MNT by all the sub-groups 2 using Asia 1 virus isolate.

The plates were stained with crystal violet stain after 48 hours of incubation on 5th day (2 November 2012). The group was taught to read the plates, and perform calculation of virus and serum titres by regression curve analysis. Later the sub-groups independently read the plates and calculated the serum SN_{50} titre of homologous and heterologous strains and also calculated the 'r' value to know the relatedness of the heterologous strain with that of reference strain.

The results are tabulated below:

Isolates	2a	2b	2c
Vaccine strain (homologous virus)	Asia 1 IND63/72	Asia 1 IND63/72	Asia 1 IND63/72
Heterologous virus	Asia1 PD271/12	PD334/12	PD334/12

SN_{50} Homologous- Heterologous- 'r' value	 2992.2 595 0.199	 3349 454 0.1355	 1948 1737 0.89

The discrepancies observed in results of 'r' value obtained among sub groups 2B and 2C was primarily attributed to handling and pipetting while performing the test, however further repetition of the test using these isolates for minimum of three times is required to compare the results.

Day-4 (1-11-12)

The works undertaken on the third day was reviewed and questions of the participants were answered. The LPBE were performed by each subgroups of Group 1 for vaccine matching on the microtitre plates coated on the previous day. Afterwards the test results were recorded.

The result of LPBE that was performed on the third day (31-10-12) revealed almost similar trends for all the groups and the 'r'-value range from 0.5 to 1 suggesting that the vaccine virus (Reference virus) is in a position to confer immunity against all the heterologous viruses tested.

Result of serotype "Asia-1" LPBE following overnight incubation at 4°C

Group	Virus	Serum Titre	r_1 Value
1a	IND63/1972	11220	
	Test Virus 1	2818	0.25
	Test Virus 2	1412	0.125
1b	IND63/1972	11220	
	Test Virus 1	2818	0.25
	Test Virus 2	1412	0.125
1c	IND63/1972	5623	
	Test Virus 1	1412	0.25
	Test Virus 2	1412	0.25
1d	IND63/1972	11220	
	Test Virus 1	1412	0.125
	Test Virus 2	1412	0.125

Result of Serotype "Asia-l" LPBE at 37°C incubation

Group	Virus	Serum Titre	r_1 Value
1a	IND63/1972	11220	
	Test Virus 1	2818	0.25
	Test Virus 2	1412	0.125
1b	IND63/1972	11220	
	Test Virus 1	2818	0.25
	Test Virus 2	1412	0.125
1c	IND63/1972	5623	
	Test Virus 1	1412	0.25
	Test Virus 2	707	0.125
1d	IND63/1972	5623	
	Test Virus 1	1412	0.25
	Test Virus 2	1412	0.25

The LPBE for vaccine matching was repeated for the serotypes Asia1 in 2 plates. One plate was kept at 4°C overnight and the other plate was incubated at 37°C for 1 hr. When the results of these two tests were compared it was observed that there was not much difference in the 'r' value. After overnight incubation the 'r' value ranged from 0.12 to 0.25. The results indicated that serotype Asia l strain IND 63/72 showed 'r' value below 0.4 and it can be assumed that the vaccine strain may not confer adequate protective immunity against the field strains tested in the preliminary screening.

Group 2: After reviewing of the previous day's work, all the subgroups of group 2 performed 2D-MNT again using serotype O homologous (R2/75) and heterologous (PD292/12 for group 2A and 292/12 for group 2B and 2C) virus. The plates were stained with crystal violet stain after 48 hours of incubation on sixth day (3 November 2012). The groups recorded the readings, calculated the titre values of virus-serum by regression curve analysis and also 'r' value to know the relatedness of the heterologous strain with that of reference strain.

The results are tabulated below:

Isolates	2a	2b	2c
Vaccine strain (homologous virus)	Type O R2/75	Type O R2/75	Type O R2/75
Heterologous virus	Type O PD369/11	PD292/12	PD292/12
$SN_{50;}$ Homologous- Heterologous- 'r' value	 2437 2371 0.972	 2985 744 0.24	 1513 1513 1.0

Day-5 (2-11-12)

Group 1: Preparation for 2D- MNT was done by all the subgroups. The BVS was diluted in serial two-fold dilutions and both the viruses were diluted at 0.5 log starting from $10^{-2.5}$. After preparing and incubating for 1 hr, cell suspension was added to the microtitre plates and kept at 37°C for 48 hrs. The 2D-MNT plates that were kept for 48 hrs were taken and stained and $TCID_{50}$ of the virus was determined. The $log_{10}SN_{50}$ titre for 100 $TCID_{50}$ was also determined. The 'r1' values of all the groups were determined. Almost all the results of all the groups were uniform despite the test being very sensitive. Another set of microtitre plates were prepared and dilutions made and kept at 37°C for 48 hrs.

'rl' values of the 2-D MNT

Group	Virus	Serum Titre	r1 Value
1a	INDR2/1975	3147	
	PD369/11	781	0.248
1b	IND63/1972	3126	
	Test Virus 1	726	0.232
1c	IND63/1972	3206	
	Test Virus 1	1306	0.407
1d	IND63/1972	4645	
	Test Virus 1	1091	0.234

Group 2: The group carried out the vaccine matching by LPBE using homologous serotype O (R2/75) and two heterologous field strains of type O. The results (serum titre) obtained are summarized below:

Isolates	2A	2B	2C
Homologous SN_{50} titre	707	707	599
Heterologous SN_{50} for V1	707	707	634
Heterologous SN_{50} for V2	707	354	713
'r' value ref:v1	1.00	1.00	1.00
'r' value ref:v2	1.00	0.5	0.6

All the three subgroups recorded `r' value of 1.00 for heterologous V1, while 'r' value above 0.4 for heterologous V2. The results indicated that both the field strains matched with the homologous virus indicating the vaccine strain presently being used covers the field strain. Further, all the three subgroups obtained similar results indicating the repeatability of the LPBE kit.

Day-6 (3-11-12)

Group 1: After reviewing the results of previous day's test, discussion to clarify doubts were held with the trainers.

Different subgroups of group I performed 2D-MNT with homologous and heterologous viruses as on fourth day (1 November 2012), and plates were kept at 37°C for 48 hrs. Results of 2D-MNT performed on fourth day were analysed and recorded. All calculations were explained by the trainers. Virus titre was estimated by Karber's method for both homologous and heterologous virus. Virus titres were expressed as $\log_{10}$ $TCID_{50}$ and serum titre as $\log_{10}SN_{50}$.

2D-MNT results

Group	Virus	Serum Titre	'r1' Value
1a	IND63/1972	3404	
	PD334/2012	641	0.188
1b	IND63/1972	2162	
	PD271/2012	1051	0.486
1c	IND63/1972	1972	
	PD334/2012	599	0.304
1d	IND63/1972	2890	
	PD271/2012	781	0.270

All the subgroups of Group 1 performed 2D-MNT for vaccine matching on two occasions independently using serotype O and serotype Asia1. For serotype O the homologous strain was OR2/75 and heterologous strain was PD-369/11. The 'r' value ranged from 0.234 to 0.407 of different subgroups.

The 2D-MNT was also conducted for vaccine matching using homologous virus (Asia1 63/72) and two heterologous strains i.e. PD334/12 and PD271/12. The results recorded that the 'r' value for PD-334/12 ranges from 0.188 to 0.304 and for PD271/12 from 0.270 to 0.486. The results were not uniform for all the groups as expected because to get an accurate 'r' value, the test must be repeated 3 times and average of the serum titres should be considered.

Group 2: The group carried out the vaccine matching by LPBE to compare antigen-antibody (Ag-Ab) incubation at two different temperatures such as at 4°C for overnight and at 37°C for one hour. The test was run using virus serotype Asia-1 63/72(homologous) and two field strains of Asia-1 (V1, V2). The results of both the protocols are presented below:

Antigen/antibody incubation at 37°C

Isolates	Gr. 2a	Gr. 2b	Gr. 2c
Homologous virus SN_{50} titre	5623	5623	5623
Heterologous v1 SN_{50} titre	1116	1412	1412
Heterologous v2 SN_{50} titre	1412	1412	707
‘r’ value ref:v1	0.198	0.25	0.25
‘r’ value ref:v2	0.25	0.25	0.125

Antigen/antibody incubation at over night at 4°C

Isolates	Gr. 2a	Gr. 2b	Gr. 2c
Homologous virus SN_{50} titre	5623	5623	5623
Heterologous v1 SN_{50} titre	1412	1412	1412
Heterologous v2 SN_{50} titre	1412	889	1412
‘r’ value ref: v1	0.25	0.25	0.25
‘r’ value ref: v2	0.25	0.16	0.25

All the groups recorded ‘r’ value of <0.4 for both the field isolates indicating that these isolates do not match with the vaccine virus. However, minimum three repetition of the test on different days are required to confirm the present observations.

Further, there was no significant difference between the two protocols (overnight incubation at 4°C and one hour at 37°C. It was concluded that any one method can be employed.

Day-7 (SUNDAY) 4-11-12

Day-8 (5-11-12)

Group 1: Following previous day’s deliberations, the groups were assigned the SN_{50} titration results to calculate SN_{50} titres and ‘r’ values to acclimatize with the calculation of the titres in 2D-MNT and plot the regression curve analysis. Each subgroup was given different values and the groups individually calculated the SN_{50} titres and ‘r’ values.

Day-9 (6-11-12)

A presentation on basic cell culture techniques was made by one of the trainer. In the presentation, he dealt with types of culture, phases of growth, basic constituent of classical media, sub-culturing of cells and GLPs for cell culture laboratory. He also covered eyeballing of the cultures. He also addressed issue of cell counting, cell viability and cryopreservation of cells. Presentation also covered the inactivation protocol of FMDV by using BEI.

The presentation was followed by open group discussion on LPBE and 2D-MNT by all the groups to clarify concepts and doubts.

Day-10 (7-11-12)

The trainees visited the different laboratories of PD-FMD and discussed various research issues pertaining to FMD virus with the laboratory in-charge scientists.

The two groups separately prepared the report on the activities carried out during the training programme and individual participants submitted the course evaluation report.

The training was concluded with feedback from each of the participants, representative from FAO as well as the trainers from PD-FMD.

The participants from Bangladesh, Bhutan, Sri Lanka and Nepal were provided with the PT reagents as well as kits for FMD diagnosis and surveillance.

Conclusions and recommendations:

During nine days training programme, the participants were introduced with the importance of FMD vaccine matching. The participants performed the vaccine matching by two-dimensional micro-neutralization (2D-MNT) test and Liquid Phase Blocking ELISA (LPBE). Barring few expected discrepancies observed in results of ‘r1’ value obtained among different sub groups, the results were uniform for the number of tests conducted. The LPBE kits have been supplied to the countries and more kits can be supplied if required. The participants submitted the evaluation report.

It is recommended that the national laboratories of every country should initiate the FMD vaccine matching using LPBE to understand the appropriateness of in-use vaccine strains in their countries. Further assistance could be provided by the Regional Leading Diagnostic Laboratory on FMD, Mukteswar, India.

Closing remarks

Dr. Venkatasubbarao Mandava, Laboratory Coordinator, Regional Support Unit/Sub Regional ECTAD, FAO, Nepal thanked SAARC member countries for deputing participants for the training. He emphasized the importance of such training that will help to build up the capacity to identify candidate vaccine strains for the country and also regional laboratory networks.

Annex 1

TRAINING AGENDA

Day 1

Time	Activities
0900-1000	Welcome and personal introduction of participants and trainers
1000-1030	Visits to Central Laboratory PD-FMD
1030-1100	Group photo, tea/coffee Break
1100-1130	Presentation: Introduction to FMD and FMDV, current status in Asia, India. Plans and preparedness in India
1130-1300	Introduction to vaccine matching and its importance. Methods/techniques for vaccine matching
1300-1400	Lunch Break
1400-1500	Collection, Preservation, and transportation of FMD suspected clinical materials
1500-1530	Laboratory practical: Processing of FMD suspected tissue/clinical materials
1530-1600	Tea/Coffee Break
1600-1700	Laboratory practical: Preparation of media, glass/plastic ware, trypsin, equipments and other reagents for cell culture -
1700-1730	Assessment for Day

DAY 2:

0930 to 1300 – Laboratory practical:

1. Sub-culturing of BHK-21 cells, Preparation of virus inoculums, and infection of healthy cell monolayer with processed clinical samples:
2. Demonstration of cytopathic effects in cell culture:
   The cytopathic changes that normally occur after infection with FMDV will be shown at different stages in comparison to the uninfected healthy cell monolayer
3. Harvesting of cell culture adopted viruses and their storage:
   (Rapid freeze thawing of the harvested infected cell culture suspension followed by centrifugation to remove cell debris and storing the virus suspension at appropriate temperature).

1400 to 1800 – Laboratory practical:

1. Titration of FMD virus in cell culture
2. Inactivation of virus using BEI for 24 hrs at 37°C and neutralized using sodium thiosulphate

3. Freeze drying of the suspension in vacuum freeze drier for 36 hrs.

Assessment for Day
DAY 3:

0930 to 1800 Trainees will be divided into two groups (group-I and group-II)

Group-I Vaccine matching by two-dimensional micro-neutralization test (2D-MNT) (Entire day with tea breaks and lunch breaks)

Theory: Principle of the test and use in vaccine matching
Laboratory:

a. Preparation of serum (BVS), vaccine virus and field isolates:
b. Virus dilution and performing 2D-MNT
c. Calculation of the serum titre that neutralizes 100$TCID_{50}$ of each virus.
d. Calculation of one way antigenic relationship (r1) value.

Group-II: Vaccine matching by LPB-ELISA (entire day with tea breaks and lunch break)

Theory: Principle of the test and use in vaccine matching
Laboratory:

a. Titration of freeze dried inactivated virus antigen and determination of optimum dilution of reagents to be used (coating and tracing serum, antigen dilutions) in the test.
b. Preparation of reagents for LPBE- Preparation of coating buffer, coating of micro-titre ELISA plates, two fold dilution of serum (BVS) and incubating along with diluted antigen in a perspex plate for overnight incubation.

Assessment for Day
DAY 4

0930 to 1800

Group-I Vaccine matching by LPBE
(Entire day with tea breaks and lunch break)

Theory: Principle of the test and use in vaccine matching
Laboratory:

a. Titration of freeze dried inactivated virus antigen and determination of optimum dilution of reagents to be used (coating and tracing serum, antigen dilutions) in the test.
b. Preparation of reagents for LPBE- Preparation of coating buffer, coating of micro-titre ELISA plates, two fold dilution of serum (BVS) and incubating along with diluted antigen in a perspex plate for overnight incubation.

Group-II: Laboratory: (entire day with tea breaks and lunch break)

Performing LPBE and taking the readings: Transfer from perspex plates to pre-coated plates, incubation and tracing with appropriate dilution of tracing serum in blocking buffer. Detection by using appropriate conjugate and substrate combination, taking the OD, determining the serum titre against different antigens.

Assessment for Day

DAY 5

0930 to 1800

Group-I; Laboratory: (entire day with tea breaks and lunch break)

1. Performing LPBE and taking the readings: Transfer from perspex plates to pre-coated plates, incubation and tracing with appropriate dilution of tracing serum in blocking buffer. Detection by using appropriate conjugate and substrate combination, taking the OD, determining the serum titre against different antigens
2. Staining of 2D-MNT plates

Group-II Laboratory: Vaccine matching by 2D-MNT
(entire day with tea breaks and lunch break)

Theory: Principle of the test and use in vaccine matching

Laboratory:

i. Preparation of serum (BVS), vaccine virus and field isolates
The stored BVS, vaccine virus and field isolates (grown to high titres) will be thawed, the serially diluted BVS will be heat inactivated at 56^0 C for 30 min.

ii. Virus dilution and performing 2D-MNT: Ten- fold diluted viruses will be incubated with serum for 1 hr at 37^0 C for neutralization and cell suspension with appropriate seeding rate will be added to wells containing neutralized viruses. Plates will be incubated for 48 hrs, observed and stained after the incubation period to calculate the serum titre for each virus.

Assessment for Day

Day 6 and Day 7:

0930 to 1800

The trainees to perform the tests as demonstrated earlier on day 3 to day 5

DAY 8

0930 to 1300

- Analysis of results
- Review results from vaccine matching round and reports

- Assessment for workshop
- Review of assessment.
- Summary of training

Concluding remarks

Annex 2

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Annex 3

Training Evaluation Sheet

We would like to assess the extent to which this training has met your expectations. Your response in the following for format will format will help us in evaluating the laboratory training programme and your understanding in the course of the training. Please mark your response and return the evaluation.

Name of Trainee and country:

Duration of the training:

SI. No.	Question	Very well Met/ very relevant/ very good/ excellent	Fully met/ relevant/ good/ very successful	Nearly met/ adequate/ successful	Yes/ fair	No/ not met/ inadequate/ poor	No response
1	The training met my expectations.						
2.	The title of the training accurately conveyed its content.						
3.	Did the programme meet the expressed objectives?						
4.	The content was organized and easy to follow.						
5.	The materials distributed were pertinent and useful.						
6.	The trainer(s) is/are knowledgeable about the subject.						
7.	The trainer(s) presented the material in an organized way.						
8.	The quality of instruction.						
9.	Was duration of programme adequate?						
10.	Class participation and interaction.						
11.	Adequate time was provided for questions and discussions.						
12.	Given the time allowed, the amount of material covered was appropriate.						

13.	Did you receive advice relevant to your work?						
14	Quality of travel arrangement received from PD-FMD during training?						
15	Quality of PD-FMD assistance?						
16	Quality of assistance received on arrival in New Delhi						
17	Quality of training programme?						
18	Quality of stipend payments, accommodation arrangements, etc?						
19	Quality of food received during training?						
20	How do you rate the training overall?						

Additional Comments: Please share with us below any additional comments or suggestions on how to improve this training.

1. What questions have been answered or solutions found during the training programme?
2. What questions, which you consider important, have been left unanswered?
3. What new questions have arisen during the course of the training programme?
4. To what extent will the training be useful in your practical professional activities?
5. How do you plan to implement it in your laboratory?
6. Your experience of social interaction in the meeting:
7. Suggestions for improvement of training programme.
8. Please note your comments and wishes.

Signature

THANK YOU FOR PARTIPITAION