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TABLE 1. Allele diversity of 36 VNTR loci for various serotype groups.

VNTR locus (alias)	Length of repeat unit (bp)	No. alleles	Range copy number ^a	Typability %	Allele diversity for serotype group:						Total (n=242)
					1a/1b/NT (n=12)	2a/2b/X/NT (106)	3a/3b (n=12)	4a/4b/Y (110)	6 (n=2)		
SF1	6	5	N, 1-4	49.6	0.50	0.02	0.64	0.02	0.00	0.55	
SF2 (ms09)	178	4	1-3, 283	100.0	0.15	0.04	0.28	0.31	0.00	0.51	
SF3 (O157-11)	6	12	2-18	100.0	0.75	0.78	0.72	0.60	0.00	0.77	
SF4	6	12	1-12	100.0	0.65	0.69	0.65	0.00	0.00	0.70	
SF5	94	6	N, 1-3, 155, 161	99.2	0.00	0.16	0.57	0.10	0.00	0.64	
SF6	9	18	N, 2-19	54.1	0.61	0.87	0.00	0.02	0.00	0.76	
SF7	5	9	2-12	100.0	0.00	0.68	0.00	0.00	0.50	0.62	
SF8 (ms22)	9	7	2-8	100.0	0.00	0.57	0.00	0.00	0.00	0.65	
SF9	9	7	1-9	100.0	0.57	0.63	0.00	0.09	0.00	0.60	
SF10	6	5	1-6	100.0	0.00	0.32	0.00	0.02	0.00	0.61	
SF11 (ms25)	17	3	1-3	100.0	0.00	0.48	0.61	0.00	0.00	0.62	
SF12 (ms07)	39	5	2-8, 389	100.0	0.00	0.00	0.50	0.05	0.00	0.54	
SF13	24	3	1-3	100.0	0.00	0.39	0.00	0.00	0.00	0.44	
SF14	12	4	1-3	94.2	0.15	0.04	0.40	0.00	0.00	0.56	
SF15	27	2	1-2	100.0	0.00	0.04	0.00	0.00	0.00	0.49	
SF16	8	2	1-2	100.0	0.00	0.00	0.50	0.00	0.00	0.50	
SF17	28	2	1-2	100.0	0.00	0.42	0.00	0.48	0.00	0.49	
SF19	6	2	1-2	100.0	0.00	0.00	0.50	0.04	0.00	0.50	
SF20	4	3	2-3	100.0	0.00	0.00	0.00	0.00	0.00	0.02	
SF21	12	3	N, 1-2	97.1	0.00	0.07	0.57	0.00	0.00	0.22	
SF22 (ms21)	141	5	N, 1-4	96.3	0.00	0.11	0.28	0.07	0.00	0.55	
SF23	7	4	N, 1-3	81.4	0.00	0.00	0.15	0.50	0.50	0.65	

SF24	18	4	3-4, 241, 258	100.0	0.00	0.02	0.50	0.02	0.00	0.53
SF25	5	9	2-11	100.0	0.00	0.00	0.79	0.02	0.00	0.10
SF26	6	4	N, 1-3	47.9	0.44	0.20	0.28	0.00	0.00	0.57
SF27	6	4	2-4, 241	100.0	0.40	0.48	0.50	0.00	0.00	0.62
SF28	10	2	1-2	100.0	0.00	0.00	0.00	0.00	0.00	0.50
SF29	3	2	3-4	100.0	0.00	0.00	0.49	0.00	0.00	0.50
SF30	8	2	2-3	100.0	0.00	0.02	0.00	0.00	0.00	0.49
SF31	6	4	2-11	100.0	0.00	0.00	0.63	0.00	0.00	0.05
SF32	6	2	1-2	100.0	0.00	0.02	0.00	0.00	0.00	0.49
SF33 (ms06)	39	3	2-4	100.0	0.00	0.11	0.00	0.00	0.00	0.15
SF34	56	3	1-3	100.0	0.00	0.48	0.00	0.00	0.00	0.49
SF35	4	2	2-3	100.0	0.00	0.00	0.00	0.00	0.00	0.50
SF36	7	3	N, 1-2	97.1	0.00	0.02	0.57	0.00	0.50	0.53
SF37	6	2	1-2	100.0	0.00	0.02	0.00	0.00	0.00	0.49

* Alleles assigned by numbers greater than 100 contain imperfect copy number of repeat unit due to deletion, insertion or composite different repeat units and are assigned by the lengths (in bp) of amplicons

TABLE 2. The discriminatory index (DI) and 95% confidence interval (CI) of various typing methods for various serotype groups

Typing method	Total (n=242)				2a (n=90)				4a/Y (n=103) ^c				Others (n=49)		
	No. types	DI	CI	No. types	DI	CI	No. types	DI	CI	No. types	DI	CI	No. types	DI	CI
PFGE	92	0.9399	0.9220-0.9578	43	0.9416	0.9096-0.9735	13	0.7158	0.6547-0.7768	37	0.9796	0.9604-0.9988			
MLVA (4) ^a	NA	NA	NA	48	0.9745	0.9643-0.9847	21	0.8654	0.8215-0.9093	NA	NA	NA			
MLVA (8) ^b	NA	NA	NA	51	0.9778	0.9682-0.9874	NA	NA	NA	NA	NA	NA			
MLVA (36)	121	0.9736	0.9632-0.9840	54	0.9800	0.9641-0.9960	26	0.8707	0.8000-0.9415	41	0.9906	0.9806-1.0001			

^aFor 2a group: SF3, SF4, SF6, SF7; for 4a group: SF2, SF3, SF17, SF23

^bFor 2a group: SF3, SF4, SF6, SF7, SF8, SF9, SF11, SF27

^cIncluding an isolate with Y serotype, which shared a common MLVA type with nine *S. sonnei* 4a isolates.

TABLE 3. Characteristics of eight *Shigella flexneri* outbreaks.

Outbreak	Year	Serotype (no. isolates)	No. of PFGE types	No. of MLVA type
A	2001/10/8 -2007/12/18	4a (102), Y (1)	13	26
B	2005/11/10 -2005/11/11	1b (6)	4	4
C	2007/9/26- 2007/10/27	2a (4)	1	1
D	2008/2/15- 2008/2/26	2a (4)	2	1
E	2005/5/9- 2005/11/4	2a (4)	1	2
H	2005/5/19- 2005/6/27	2a (7)	1	1
I	2005/10/22- 2005/10/24	2a (5)	1	1
K	2008/3/27- 2008/4/4	2b (1), X (10)	4	6

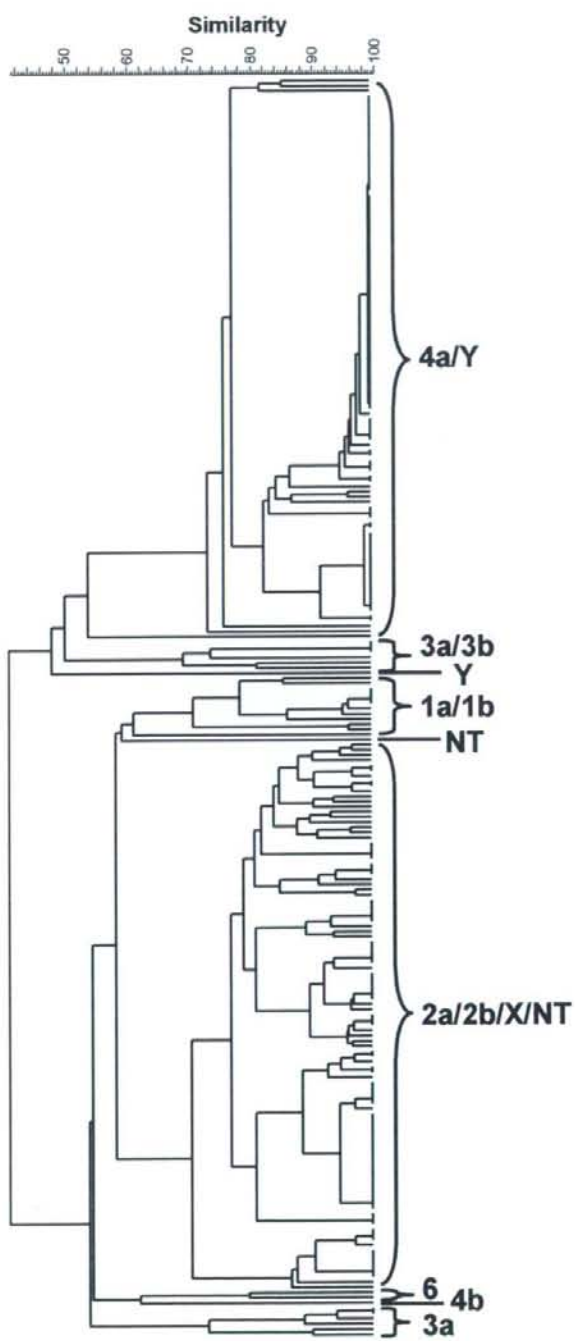


FIG. 1. Dendrogram constructing using PFGE patterns for 242 *Shigella flexneri* isolates. Groupings were defined on the basis of the level of genetic relatedness and serotypes.

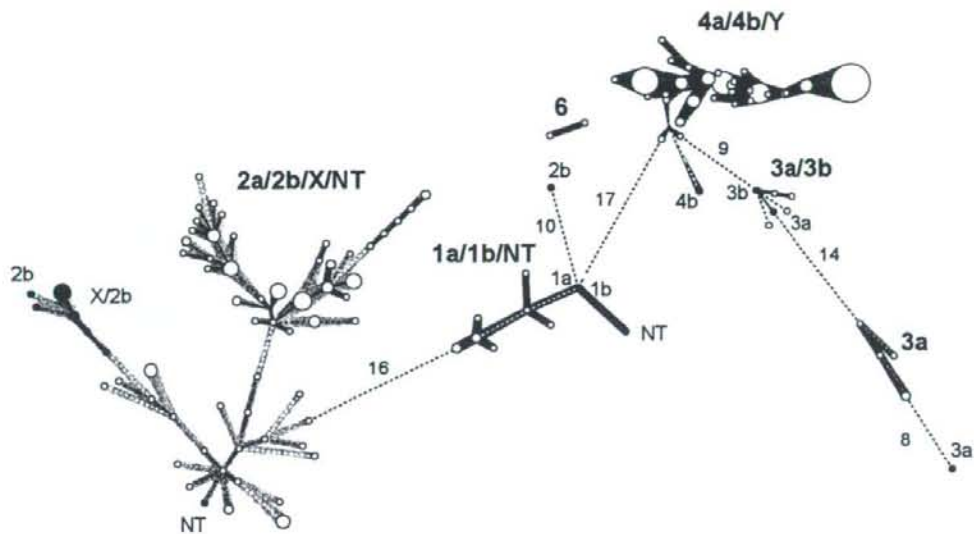


FIG. 2. Minimum spanning tree constructed using the MLVA genotypes for the 242 *Shigella flexneri* isolates. A clonal group includes genotypes differing at seven or fewer loci from the closest one. There are six clonal groups designated in bold characters. Circles in red and blue are marked with the serotype or subserotypes. Distances (in number of loci) between clonal groups or singletons are numbered. Circle size is proportional to the number of isolates belonging to the indicated MLVA genotype.

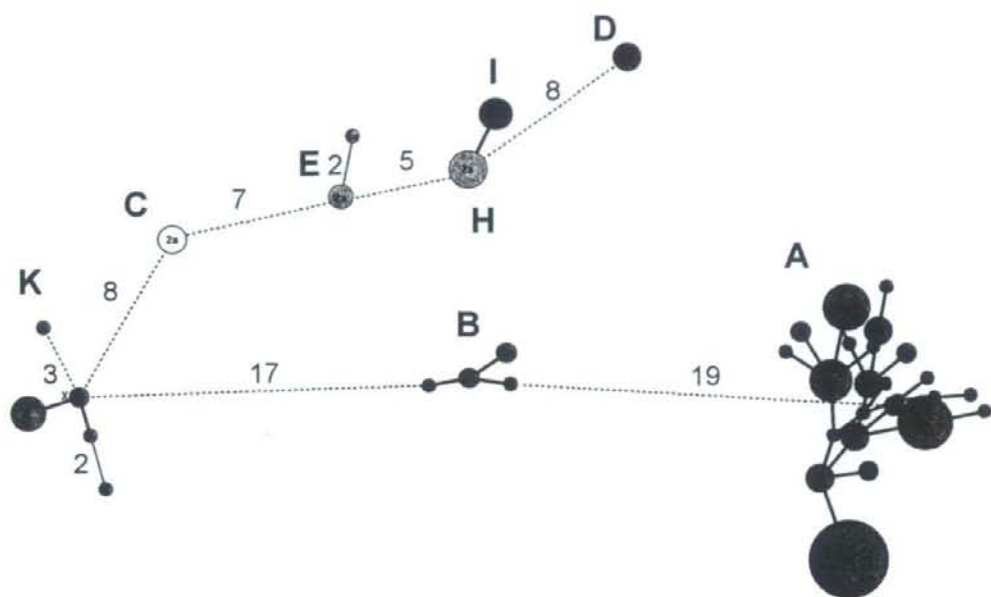


FIG. 3. Minimum spanning tree constructed using the MLVA genotypes for the *Shigella flexneri* isolates collected from 8 shigellosis outbreaks. Each of the outbreaks is indicated by different colors. The serotype or subserotype for isolate(s) of the MLVA type is marked inside the circle. Circle size is proportional to the number of isolates belonging to the indicated MLVA genotype. Differences in two or more loci between two MLVA types are numbered.



**ASEAN PLUS THREE
EMERGING INFECTIOUS DISEASES PROGRAMME**

**ASEAN PLUS THREE PROTOCOL
FOR LABORATORY BASED SURVEILLANCE (LBS)
OF SELECTED PATHOGENS**

August 2008

ASEAN PLUS THREE EMERGING INFECTIOUS DISEASES PROGRAMME

ASEAN PLUS THREE PROTOCOL FOR LABORATORY BASED SURVEILLANCE (LBS) OF SELECTED PATHOGENS

1. INTRODUCTION

The Protocol is the output of the ASEAN Plus Three Workshop on Development of Laboratory Based Surveillance (LBS) and Strengthening Regional Laboratory Networking, held on 11-13 August 2008 in Kuala Lumpur, hosted by the Ministry of Health of Malaysia, under the ASEAN Plus Three Emerging Infectious Diseases Programme Phase II.

The aim of the protocol is to enhance, strengthen, harmonise and operationalise the laboratory-based surveillance on selected infectious pathogens which are of concern to public health in this region. Monitoring their activities is hoped to contribute to early intervention and reduce the impact of these pathogens.

A system for laboratory based surveillance in this region had been recognised by ASEAN Member States (AMS) and Plus Three Countries (China, Japan, Republic of Korea) during the previous ASEAN+3 / WHO workshops held in Kuala Lumpur, Malaysia. Thirteen infectious pathogens were selected by consensus for the Laboratory Based Surveillance based on criteria including the outbreak potential, impact on health as well as socio-economic development in the region,

The initial selected 13 infectious pathogens are as follows:

Dengue virus, Enterovirus 71, Hepatitis E virus, Hepatitis A virus, Japanese encephalitis virus, *Vibrio cholerae*, *Neisseria meningitidis*, *Escherichia coli* 0157:H7, *Corynebacterium diphtheriae*, *Bordetella pertussis*, *Haemophilus influenzae* type b, Methicillin resistant *Staphylococcus aureus* (MRSA) and malarial parasite.

The Standard Operation Procedures (SOPs) for carrying laboratory diagnostic tests for the selected pathogens were extensively discussed and revised accordingly in the 4th ASEAN+3/ WHO Workshop held in Kuala Lumpur in November 2006. The final documents were accepted by general consensus.

The sharing of surveillance data on these 13 pathogens as part of the Protocol for Communication and Information Sharing on Emerging Infectious Diseases in

the ASEAN Plus Three Countries has been endorsed by the ASEAN Expert Group on Communicable Diseases (AEGCD) in December 2007, and adopted during the 4th Senior Officials' Meeting on Health Development (SOMHD) in December 2007 in Vientiane, Lao PDR.

The ASEAN Plus Three Protocol for Laboratory Based Surveillance will provide a platform for future expansion (not limited to only 13 pathogens), and integration from others sources of laboratory data and information sharing (eg. data from animal health, food and environment).

2. PURPOSE OF THE PROTOCOL

The purpose of the protocol is to provide guidelines and standard methods for the operationalisation of LBS system for selected infectious pathogens in the region.

3. OBJECTIVE OF LABORATORY BASED SURVEILLANCE IN THE REGION

3.1 General Objective

To conduct LBS on the selected pathogens circulating in the region.

3.2 Specific Objectives

1. To share information on and monitor the selected pathogens circulating in the region
2. To share information on and monitor virulent/ variant/ novel strain/ antimicrobial resistance if applicable
3. To assist in predicting outbreaks and/or providing early warnings; and
4. To share information on and monitor zoonotic pathogens (future)

4. STRATEGY OF LABORATORY BASED SURVEILLANCE IN THE REGION

1. Each of the ASEAN Plus Three Countries' existing surveillance or monitoring system of the pathogens will be used.
2. The background information on the LBS system from each ASEAN Plus Three Country or each pathogen under surveillance will be collected in order to further understand each country's data limitation.
3. Data collection and reporting will be done through a web- based platform, i.e, <http://www.aseanplus3-eid.info>

4. Passive surveillance of the selected pathogens will be employed, and data uploading will be on a voluntary basis using the principle of trust, confidence, and information sharing within the region.
5. Enhancement of regional LBS will be accomplished through strengthening of each country's laboratory capacity and capability by means of funding and training where necessary.

5. SCOPE OF LABORATORY BASED SURVEILLANCE IN THE REGION

The initial 13 selected pathogens from human source selected for surveillance are:

5 viruses

Dengue virus, Enterovirus 71, Hepatitis E virus, Hepatitis A virus, Japanese encephalitis virus.

7 bacteria

Vibrio cholerae, *Neisseria meningitidis*, *Escherichia coli* 0157:H7, *Corynebacterium diphtheriae*, *Bordetella pertussis*, *Haemophilus influenzae* type b, Methicillin Resistant *Staphylococcus aureus* (MRSA),

1 parasite

Malaria parasite (*Plasmodium* spp.)

Future expansion (more pathogens) and integration (with food, animal and environment source) for LBS will be further identified by ASEAN Plus Three Countries through workshops and networking.

6. METHODOLOGY FOR THE SURVEILLANCE

All ASEAN Plus Three Countries are requested to upload the data on the selected pathogens on a regular agreed periodicity.

6.1 Type of surveillance

Laboratory based surveillance

6.2 Population under surveillance

Each of the ASEAN Plus Three Countries is to provide background information on the laboratory surveillance system of the selected pathogens, to be updated as necessary- **Appendix 2.**

6.3 Data/ Information to be collected

The following data and information are to be collected using the agreed form on 'ASEAN Plus Three Laboratory Based Surveillance Monthly Report'- **Appendix 3.**

- Number of clinical cases*
- Number of laboratory confirmed cases*
- Number of specimens tested* (When more than one specimen is taken from one patient, the total number of specimens is to be recorded as such)
- Number of pathogens isolated / detected by specific method* (To refer to ASEAN Plus Three SOP for each pathogen if available)
- Monthly comment In addition to the data agreed, ASEAN Plus Three Countries are encouraged to share information on outbreak occurrence (may include outbreaks due to any pathogen), Virulent / variant / novel strain/ antimicrobial resistance encountered, imported cases or any information deemed relevant by the country.

** When no cases are found, they are to be filled as '0', and where tests were done but no positive result found, this is also to be filled as '0'.*

6.4 Frequency of data collection

The agreed data are to be collected on a monthly basis (updated within the following month)

6.5 Monitoring Reports (aggregated report) – **Appendix 4 I, ii, iii, iv and v.**

Tabulated reports will be auto-generated by the system based on each country's monthly report, and will be accessible to registered members on the website (<http://www.aseanplus3-eid.info>).

6.6 Outbreak detection

In addition to the data agreed, ASEAN Plus Three Countries are encouraged to share information on outbreak occurrence (may include outbreaks due to any pathogen) in order that the data may be available for use in risk assessment and outbreak prediction.

7. SURVEILLANCE SYSTEM MANAGEMENT

7.1 Data collection from the source will be the responsibility of each ASEAN Plus Three Country. Each country's NLCP is to collate, verify and validate data to be uploaded to the <http://www.aseanplus3-eid.info> in consultation with the Communication Focal Point of each country as necessary – **Appendix 5.**

7.2 ASEAN Plus Three Countries' National Laboratory Contact Point (NLCP) to update data in the website monthly.

7.3 Data security

7.3.1 Data is web- based and password protected (registered user) and is to be restricted to the registered users only.

7.3.2 ASEAN Secretariat and website manager (Indonesia) will employ measures to ensure data security.

7.4 Data ownership

The data uploaded by each country is owned by the respective country and any use of the data for any purpose (such as publication, presentations, etc) will need to be cleared through an official approval from the country that owns the data. The country is able to review, revise the uploaded data of the respective country at any time, as necessary.

7.5 Accessibility

Web based through <http://www.aseanplus3-eid.info>

7.6 Laboratory Based Surveillance system management

The overall smooth functioning of the laboratory based surveillance will be the responsibility of a Core Group who will also be responsible for regular reporting to the ASEAN Plus Three Countries on the progress of the regional laboratory based surveillance. The Core Group will be comprised of at least the following:

1. ASEAN Secretariat will be responsible for overall management (policy, monitoring etc);
2. Indonesia will be responsible as website manager for upgrading of technical aspect of website as required for laboratory aspect; and
3. Malaysia will be responsible for future expansion of laboratory surveillance aspect such as animal health, etc.

WHO and other relevant technical organizations are to be invited to be members of the Core Group as appropriate.

7.7 User Manual

The Core Group for Laboratory Based Surveillance will develop the user manual as reference to be distributed to all the ASEAN Plus Three Countries

7.8 Training needs

Any training needs as expressed may be accomplished through development of training manual or as appropriate by the Core Group for Laboratory Based Surveillance.

ASEAN Plus Three Partnership Laboratories (APL)

Terms of Reference

A. Objectives

General Objective

To strengthen the capacity of ASEAN Plus Three Countries to appropriately respond to infectious diseases through regional laboratory networking.

Specific Objectives

1. To establish and operationalise the ASEAN Plus Three regional network of health laboratories to support rapid communication and information sharing, regional laboratory-based surveillance and capacity building.
2. To strengthen the health laboratories' diagnostic capacity, quality and biosafety through sharing of resources among the ASEAN Plus Three Countries.
3. To promote¹ research on infectious diseases in the region through cooperation and collaboration among health laboratories.

¹ To bring up during regional APL meeting: agreements for sharing of specimens, material transfer??

B. Characteristics of APLs

1. The participation of the country's health laboratories in the region as ASEAN Plus Three Partnership Laboratories (APLs) is on voluntary basis.
2. Ability to perform and support definitive identification and characterization of pathogen especially of novel strain or type that emerges in this region.
3. Ability to support and supply reference standards/reagents for diagnostic testing and proficiency testing in regional laboratory quality assurance system.
4. Ability to support and provide technical laboratory training to strengthen country laboratory diagnostic capability.
5. Ability to achieve quality diagnostic output
6. Ability to give appropriate feedback and turn around time.
7. The designated APLs have the privilege of using the APL logo to reflect the identity of the regional system of partnership laboratories.
8. All national laboratory contact points (NLCP) shall be automatically designated as APLs to serve as the respective country laboratory resource centre, as rapid communication link related to laboratory issues, and as respective country coordinator of laboratory-based surveillance.
9. APLs shall be responsible for mobilising funds for their own activities.

C. Nomination, Selection and Review Process

1. Laboratories in each country could apply to the Ministry of Health to be nominated as an APL, through the NLCP, using the application form provided by the APL Steering Committee, with supporting documents.
2. The candidate APL of each country shall be nominated by the Ministry of Health through the NLCP.
3. The nomination shall be submitted to the APL Steering Committee for evaluation.
4. ASEAN Secretariat will issue official appointment letters to respective designated APLs, based on decision of AEGCD/ PCG.
5. Upon designation, the APL agrees to perform the responsibilities as outlined in this document.
6. The APL Steering Committee shall review the status of existing APLs initially every 3 years and make the necessary recommendations to the AEGCD/ PCG on redesignation.

D. Activities of ASEAN Plus Three Partnership Laboratories

1. Perform definitive identification and characterization of pathogen especially of novel strain or type that emerges in this region
2. Provide support to other health laboratories in the region for definitive identification and characterization of pathogen especially of novel strain or type that emerges in this region
3. Support and supply reference standards/reagents for diagnostic testing and proficiency testing in regional laboratory quality assurance system within the limits of available resources (funding)
4. Support or facilitate technical laboratory training to strengthen country laboratory diagnostic capability.
5. Provide annual report on its activities in accordance to its characteristics to the NLCP for submission to Steering Committee, and to AEGCD and counterparts of the Plus Three Countries.
6. APLs are encouraged to host a periodic scientific and operational meeting of APLs to strengthen regional infectious disease laboratory diagnostics, networking, collaboration in research and development of new diagnostic tests.

E. APL Steering Committee

1. The APL Steering Committee will be established, and managed by the AEGCD through the Programme Facilitation Section of the Health and Communicable Diseases Division of the ASEAN Secretariat.
2. The membership and experts for the Steering Committee shall be endorsed for a period of three years by the AEGCD.
3. The Committee shall consist of :
 - a. 2 WHO representatives (1 from SEARO and 1 from WPRO),
 - b. 2 regional bacteriologists representing ASEAN +3 Countries
 - c. 2 regional virologists representing ASEAN+3 Countries
 - d. 1 regional parasitologist representing ASEAN+3 Countries
 - e. 1 regional mycologist representing ASEAN+3 Countries
 - f. 2 regional veterinary laboratory experts in zoonosis (1 bacteriology, 1 virology) representing ASEAN+3 Countries
 - g. 1 representative of ASEAN Secretariat (Health and Communicable Diseases Division)
 - h. 1 representative of AEGCD
4. The Committee members will be decided upon by the AEGCD.

5. The Chair of the APL Steering Committee will be elected from within the Committee, and will have a casting vote in the case of tied decisions. The Chair will have a term of one year, renewable.
6. The Committee shall be supported by the Programme Facilitation Section.²
7. The APL Steering Committee will convene a meeting at least once a year or as necessary.
8. The APL Steering Committee shall review and select the proposed laboratories and recommend them to the PCG, for designation as APLs.
9. The APL Steering Committee shall review the status of existing APLs initially every three years and make the necessary recommendations to the AEGCD on redesignation.
10. Members of the APL Steering Committee are encouraged to attend the periodic scientific and operational meetings of APLs to strengthen regional infectious disease laboratory diagnostics, networking, collaboration in research and development of new diagnostic tests.

F. Operationalising the APL and its sustainability

1. Uploading
 - a. The list of APLs shall be uploaded onto the ASEAN Plus Three EID website (www.aseanplus3-eid.info) by the country responsible for the website (Indonesia) in coordination with the ASEAN Secretariat through the Programme Facilitation Section.
 - b. The list shall be uploaded/ updated annually after annual APL Steering Committee meeting.
2. Publication

A directory of APLs shall be prepared and distributed annually by the Programme Facilitation Section.
3. Establishing and maintaining contact with other laboratories
 - a. A designated APL may directly communicate and conduct activities with other APLs as they wish, in accordance with national policies.
 - b. A non-APL may also directly communicate and collaborate with an APL, and vice versa, in accordance with national policies.
 - c. APLs shall be responsible for mobilising funds for their own activities.

² Consider a secretariat for the Committee