



**National Health Research Institutes
(NHRI), Zhunan, Taiwan**



**Children's Hospital No. 1 (CH1),
Ho Chi Minh City (HCMC), Vietnam**

International collaboration between NHRI, Taiwan and CH1, Vietnam

Min-Shi Lee, MPH, PhD

Background

- Distance between CH1 and NHRI: 2153 Km, tropical and subtropical
- Population: 23M in Taiwan, 96M in Vietnam and 8.6M in HCMC
- Neonates: 1.6M in Vietnam
- CH1-HCMC: 1600 beds, 6000 visits/day, 100K admissions/year





Collaborations Between CH1-HCMC and NHRI-Taiwan, 2000~2010 (NHRI PI: Dr Ih-Jen Su)

2000 ~2006 (pilot studies)

I

EBV-associated
Hodgkin's
lymphoma

II

Hemophagocytic
Syndrome

III

Immunopathogenesis
of Dengue Fever (5
ppapers)

Collaborative Project



2006 ~ 2010 (Official Contract)

I

Established Molecular
Diagnosis Laboratory in
CH1 (PLoS One 2013)

II

Milrinone Trial for EV71
Lung Edema (Critical
Care Med 2013)

III

EBV-Hemophagocytic
Syndrome (Br J
Haematol 2010)

Training Program

2005 year

Heart Surgery

NHRI offered a training program for **Dr. Nguyen Duc Tuan, Dr. Nguyen Thi Tran Chau and Dr Do Nguyen Tin** at the **NCKU Hospital** to participate open heart surgery.

2008 year

Virus Isolation

NHRI offered a training program for **Dr. Le Quoc Thinh and Ms Nguyen Ngoc Viet Nga** at the **NHRI Laboratory** to participate virus isolation training program.

2008 year

Molecular Genetic

NHRI offered a training program for **Mr. Bui Vo Minh Hoang** at **Chi Mei Medical Center** to participate molecular genetics training program.

PI: Prof. Ih-Jen Su



蘇益仁 特聘研究員

國家衛生研究院

2009

CH1 Virology Lab was launched on
12 Nov 2009 with funding from NHRI,
Taiwan



Prof. Ih-Jen Su received a HCM Medal in 2009

蘇益仁特聘研究員安排越南第一兒童醫院心臟外科團隊在統一企業高鴻源董事長的支助下，到台南成大醫院接受為期六個月的訓練，終於成功開展該院的小兒開心手術，目前已進行上千個小兒的手術。於2009年獲頒越南政府胡志明獎及胡志明市榮譽市民，表彰其長期投入越南第一兒童醫院病毒研究的貢獻。



CH1-NHRI Collaboration Since 2011



2011~Present

Hospital-based Surveillance of
EV-A71 in HCM City, Vietnam

2015~Present

Hospital-based Surveillance of
Influenza in HCM City,
Vietnam

2017~Present

Establishment of Asia-Pacific
Network for Enterovirus
Surveillance (APNES)

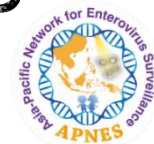
NHRI PI: Dr.
Min-Shi Lee

Species and Serotypes of Human Enterovirus International Committee on Taxonomy of Viruses (ICTV), 2017

<i>Species</i>	No. of serotypes	Serotypes
<i>Enterovirus A</i>	25	EV-A71 , CA2-8, CA10, CA12, CA14, CA16, EV-A89-91, EV-A114, EV-A119, EV-A120, EV-A121, SV19, SV43, SV46, BA13
<i>Enterovirus B</i>	63	CA9, CB1-6, E1-7, E9, E11-E21, E24-27, E29-33, EV69, EV73-75, EV77-88, EV93, EV97-98, EV100-101, EV106-107
<i>Enterovirus C</i>	23	PV1-3, CA1, CA11, CA13, CA17, CA19-22, CA24, EV-C95-96, EV-C99, EV-C102, EV-C104-105, EV-C109, EV-C113, EV-C116
<i>Enterovirus D</i>	5	EV-D68, EV-D70, EV-D94, EV-D111, EV-D120

EV-A71 can be classified into 3 major genogroups (A, B and C) including 11 genotypes (A, B1~B5, C1~C5).

SEVERE ENTEROVIRUS INFECTIONS IN TAIWAN SINCE 2012



台灣地區腸病毒重症偵測

Year	Suspected Cases	Confirmed Cases (deaths)	EV Serotypes (cases no.)
2018	116	18 (4)	Echo11(8), CV-A4(2), CV-B1(2), EV71(2), CV-A9, CV-A16, CV-B2, CV-B3
2017	103	23 (1)	EV-D68(11), CV-A6 (3), CV-A9 (2), CV-B3 (2), Echo5 (2), EV71 (2) , CV-A2 (1)
2016	142	33 (1)	EV-71 (23) , CA4 (2), CA6 (2), CB3 (2), CV-A2 (1), CA5 (1), CB4 (1), Echo18 (1)
2015	100	6 (2)	CV-B5 (3), CA16 (2), Echo3 (1)
2014	54	6 (1)	CVA2, CVA5, CVA16, CVB5, Echo11, EV71
2013	122	10 (1)	EV71 (6) , CA6 (3), Echo30(1)
2012	294	146 (2)	EV71 (139) , EV-71+CA2 (1), EV-71+CB3 (1), EV-71+CB4 (1), EV71+Adeno (1), CV-A2 (2), Echo (1)
合計			

Data Source: Taiwan CDC

Top five enterovirus serotypes in Taiwan, Since 2005 (Taiwan CDC)						
Year	No. of Enterovirus isolates (Medical visits 、 classes suspended)	Top 1	Top 2	Top 3	Top 4	Top 5
2005	1924	CV-B3 37%	CV-A16 24%	EV-A71 18%	CV-A6 6%	CV-A5 4%
2006	1303	CV-A2 19%	CV-A5 15%	CV-A4 15%	ECHO-18 15%	CV-B2 10%
2007	856	CV-A6 25%	CV-A10 21%	CV-A16 19%	CV-A4 7%	ECHO-6 5%
2008	670	CV-A2 34%	EV-A71 27%	CV-B4 11%	CV-A16 4%	CV-B1 3%
2009	1431	CV-A6 29%	CV-A4 25%	CV-A6 17%	CV-A5 12%	CV-A5 5%
2010	1742	CV-A16 30%	CV-A4 20%	CV-A6 17%	CV-A5 12%	CV-B1 3%
2011	3315	CV-A10 45%	CV-A9 18%	CV-A4 9%	CV-B5 9%	EV-A71 9%
2012	2250 (505590, 12156)	EV-A71 39%	CV-A2 19%	CV-B3 12%	CV-A4 7%	ECHO-6 6%
2013	1463 (605663, 17116)	CV-A16 31%	CV-A4 9%	CV-B4 8%	CV-B2 6%	CV-A10 5%
2014	1147 (522811, 20186)	CV-A10 32%	CV-A4 19%	CV-B4 8%	CV-B2 6%	CV-A9 8%
2015	1036 (555618, 22576)	CV-A16 32%	CV-A4 16%	CV-A6 15%	CV-A5 10%	ECHO-6 3%
2016	1843 (611959, 28599)	CV-A10 29%	CV-A5 18%	CV-A2 16%	CV-A4 7%	CV-A16 5%

RESEARCH ARTICLE

A Molecular Approach Applied to Enteroviruses Surveillance in Northern Taiwan, 2008-2012

Wan-Yu Chung¹, Pai-Shan Chiang¹, Shu-Ting Luo¹, Tzou-Yien Lin^{2,3}, Kuo-Chien Tsao^{4,5}, Min-Shi Lee^{1*}

1 National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes (NHRI),

Table 1. Annual positive rates for enterovirus detection by two different laboratory methods, 2008–2012.

Year	Virus isolation		VP1-CODEHOP test	
	n/N	%	n/N	%
2008	18/61	29.5%	22/61	36.1%
2009	21/49	42.9%	26/49	53.1%
2010	89/160	55.6%	112/160	70.0%
2011	40/86	46.5%	47/86	54.7%
2012	40/75	53.3%	43/75	57.3%
Total	208/431	48.3%	250/431	58.0%

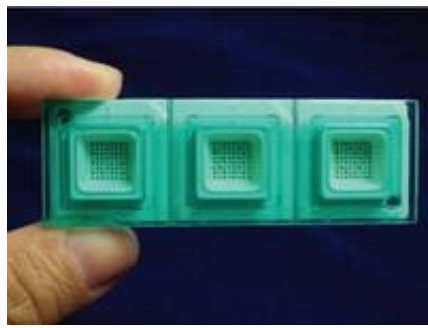
doi:10.1371/journal.pone.0167532.t001

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Data Availability Statement: All relevant data are within the paper and its Supporting Information

and two showed consistent serotypes with the VP1-CODEHOP and VI-IFA tests, respectively. CVA16, CVA6 and EV71 were the most prevalent serotypes in northern Taiwan, 2008–2012. Moreover, variant CVA2, CVA6 and EV71 viruses were further identified based on phylogenetic analysis of partial VP1 sequences. In conclusion, the VP1-CODEHOP test could be used as the primary method for enterovirus surveillance to support decision-making for outbreak control.





晶字生物科技
DR. Chip BIOTECH

- DIY kit from Dr. Chip Biotech
 - A ready to use polymer substrate and colorimetric reagents system
 - The kit provides a complete set of reagents for making microarray from **probe immobilized, hybridization, and blocking to color signal** reaction
 - HPV, MTBC
- Immobilize designed DNA probes
- Read with naked eyes



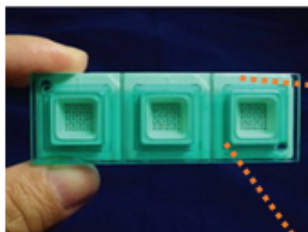
PCR 1 ~750bp

PCR 2 ~350bp

GCAATGCTGGGTACACACGTCATCTGG...TCCAGTATATGTTTGTTCCTCCCTGGCG

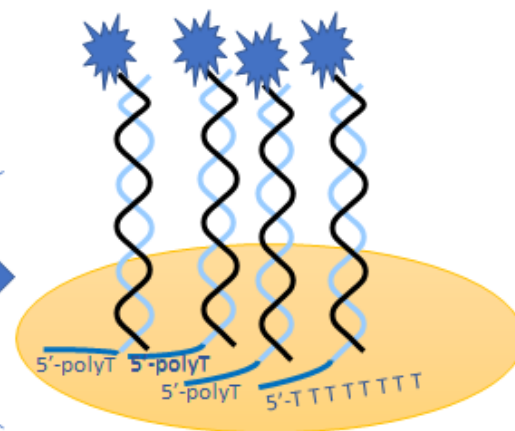
GCTGCTGAAATTGGGGCATCGTCAAAT...TTACTTCAGTATATGTTTGTTCCTCCCT
GCTGCTGAGATCGGGGCATCGTCAAAT...CTACTCCAGTACATGTTTGTTCCTCCCT
GCCGCTGAAATTGGAGCATCGTCAAAT...TTGCTCCAATACATGTTTGTGCCACCT
GCCGCTGAAATTGGAGCATCATCAAAT...TTGCTCCAATATATGTTTGTACCACCT
GCTGCTGAAATTGGGGCATCGTCAAAT...TTACTCCAGTATATGTTTGTTCCTCCCT

●-Biotin
★-Blue precipitate



20170904 5th batch probes

	1	2	3	4	5	6
A	A/EV71 (2probe)	A/EV71 (2probe)	A/EV71	B/E30 (degenerate)	B/CVB3 (2probe)	Human-NHRI
B	A/CA16 (3probe)	A/CA6 (2probe)	PanA/CA4 (2probe)	B/E30	B/CVB3 (3probe)	C/PV1 (2probe, VDP V)
C	A/CA2 (2probe)	A/CA2 (only old)	PanA/CA4 (2probe)	B/E30	B/CVB5 (2probe)	C/PV1
D	A/CA2 (only new)	A/CA2	PanA/CA4 (2probe)	PanA/CA5 (3probe)	B/CVB5 (3probe)	C/PV1 (3probe, wild-type)
E	PanA/CA10 (2probe)	PanA/CA10	PanA/CA10 (2probe, VN+TW)		PanC/CA24 (2probe)	PanC/PV2 (3probe)
F	HC	D/EV-D68 (2probe) 3 rd design	D/EV-D68 (2probe) 4 th design	PanC/CA24 (2probe)	PanC/CA24 (2probe)	PanC/PV3 (degenerate)

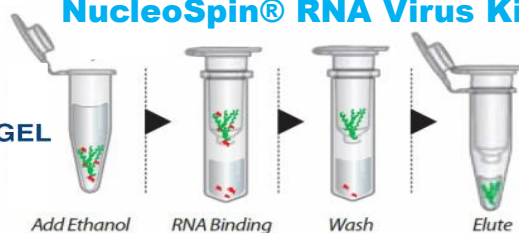




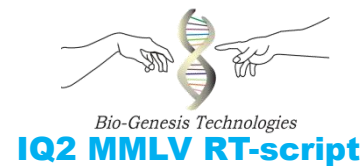
BD universal viral transport system



NucleoSpin® RNA Virus Kit



RNA extraction



An32, An33, An34, An35

PCR CODEHOP1



Chip Test

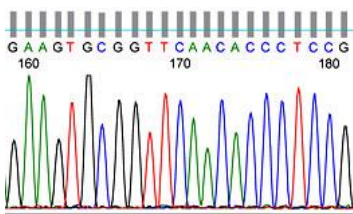
Q-Amp™ 2x Screening Fire Taq Master Mix



An88, An89

Sequencing

提供晶片相關技術指導



MB MISSION BIOTECH

明欣生物科技有限公司



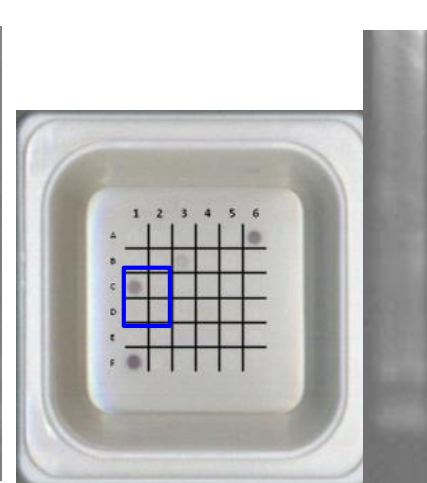
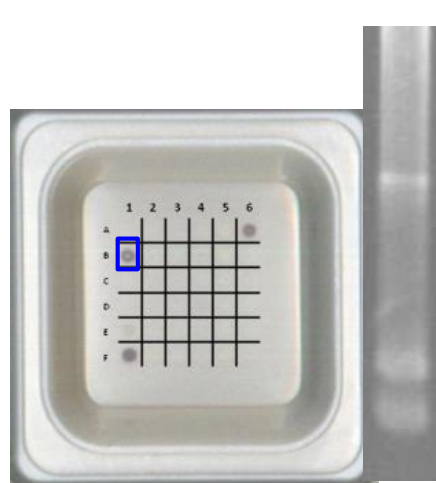
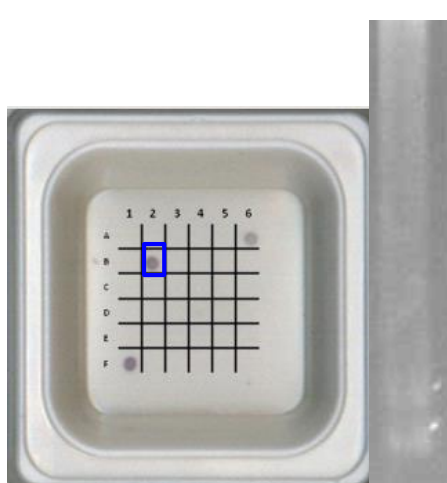
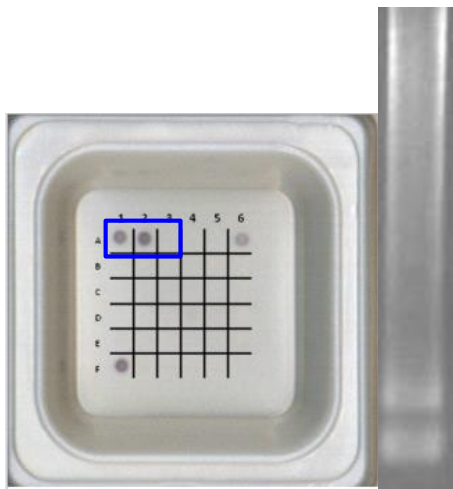
Q-Amp™ 2x Screening Fire Taq Master Mix

Nested-PCR CODEHOP2



222, 224





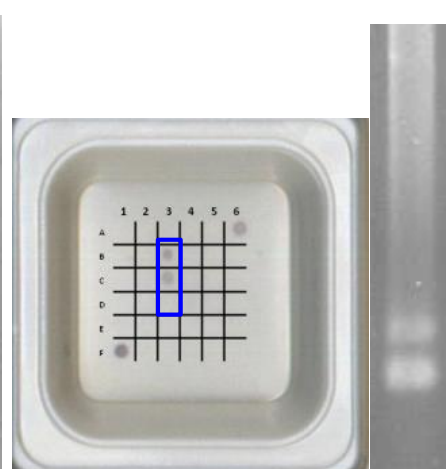
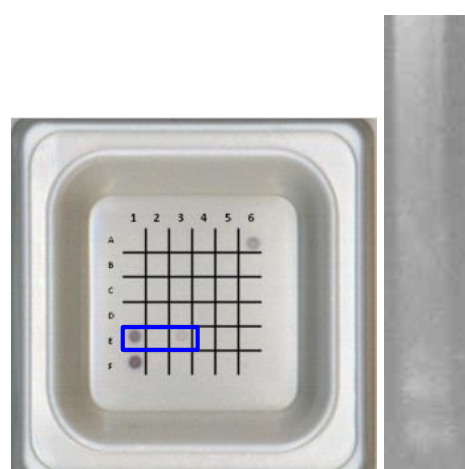
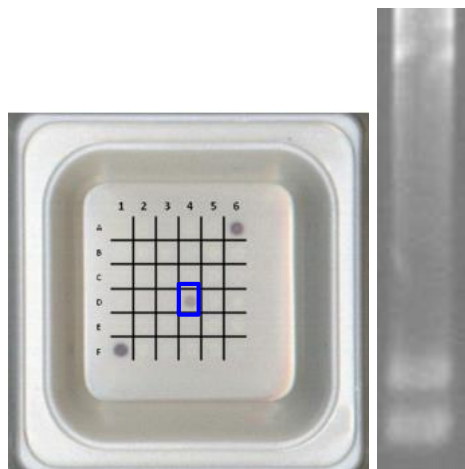
PCR1

PCR2

Beta-g

2016-E474 / EV71/C4 2014-E346 / CA6 2016-E371 / CA16 2016-E312 / CA2

↳ Determined by sequencing VP-CODEHOP product



2016-E447 / CA5

2015-E294 / CA10

2013-E170 / CA4

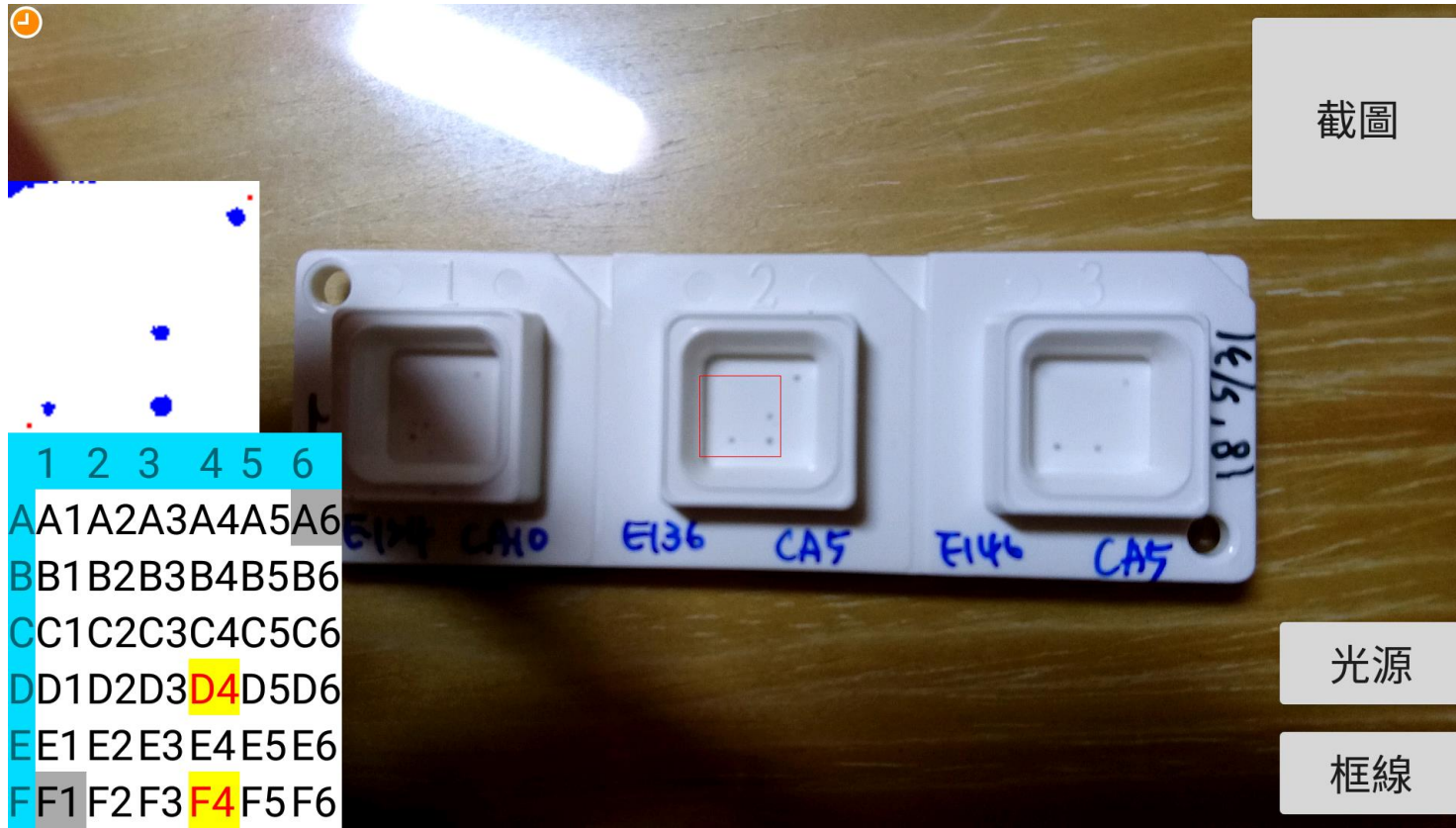
20170904 5th batch probes

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B	A/CA16 (3probe)	A/CA6 (2probe)	PanA/CA4 (2probe)	B/E30	B/CVB3 (3probe)	C/PV1 (2probe, VDP V)
C	A/CA2 (2probe)	A/CA2 (only old)	PanA/CA4 (2probe)	B/E30	B/CVB5 (2probe)	C/PV1
D	A/CA2 (only new)	A/CA2	PanA/CA4 (2probe)	PanA/CA5 (3probe)	B/CVB5 (3probe)	C/PV1 (3probe, wild- type)
E	PanA/CA10 (2probe)	PanA/CA10	PanA/CA10 (2probe, VN+TW)		PanC/CA24 (2probe)	PanC/PV2 (3probe)
F	HC	D/EV-D68 (2probe) 3 rd design	D/EV-D68 (2probe) 4 th design	PanC/CA24 (2probe)	PanC/CA24 (2probe)	PanC/PV3 (degenerate)



FUTURE DIRECTIONS

- To resolve untypable and equivocal problems
 - Renewing probes
 - Using whole-genome NGS platform
- To develop smart phone App
- To perform clinical trial



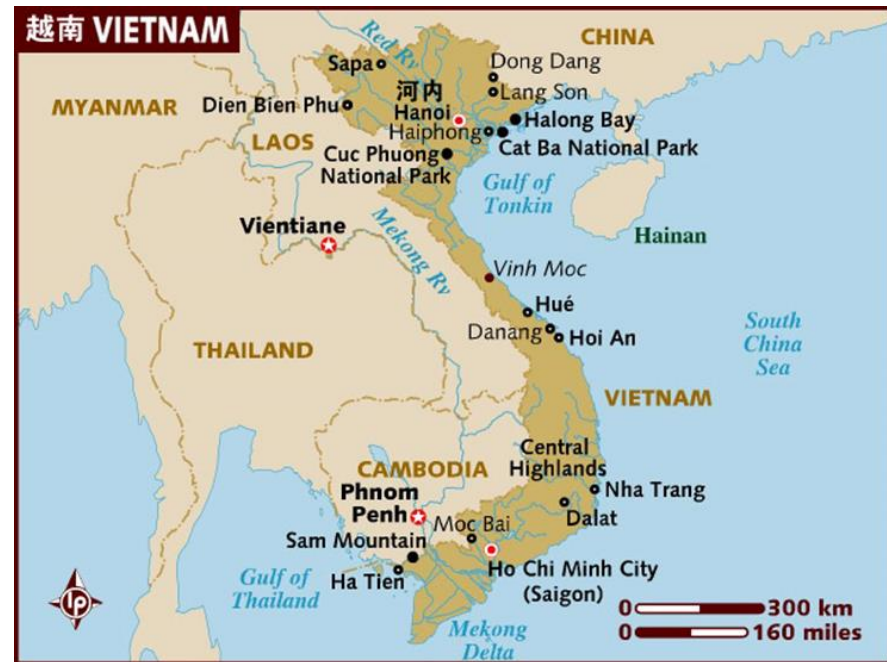


Hospital-based Surveillance of EV-A71 in HCM City, Vietnam, 2011-2017

Enterovirus in Vietnam

- ◆ In Vietnam, EV-A71 was first identified in 2003.
- ◆ A large EV-A71 epidemic was documented in southern Vietnam in 2005. (173 EV-A71 cases including 51 with neurological complications and 3 fatal cases.)
(Phan Van Tu et al., 2007)
- ◆ From 2006 to 2010, only sporadic EV-A71 cases were detected in southern Vietnam.
- ◆ In 2011, EV-A71 epidemics occurred again in southern Vietnam.

(Khanh TH et al., 2012)



Material and Methods

Flowchart of Hospital-Based Surveillance of EV-A71 in Vietnamese Children

Epidemiology of HFMD

(hand, foot, mouth and disease)

HFMD inpatients (~30 cases/month)

Throat swab collection
3ml/tube (2 tubes)

Serum collection
2ml/vial

Virus culture in
CH1 virology lab

Frozen at -70°C and
shipped to NHRI,
Taiwan

Throat swabs
were used for
virus isolation.

Throat swabs were
used for genotyping
using molecular
test (CODEHOP)

Sera were used
to measure
neutralizing
antibody
against EV-A71

Seroprevalence of EV-A71 Antibody

Non-enterovirus-related inpatients such as
allergy or chronic disease, ~30 cases/month,
2012-2013

Serum collection
2ml/vial

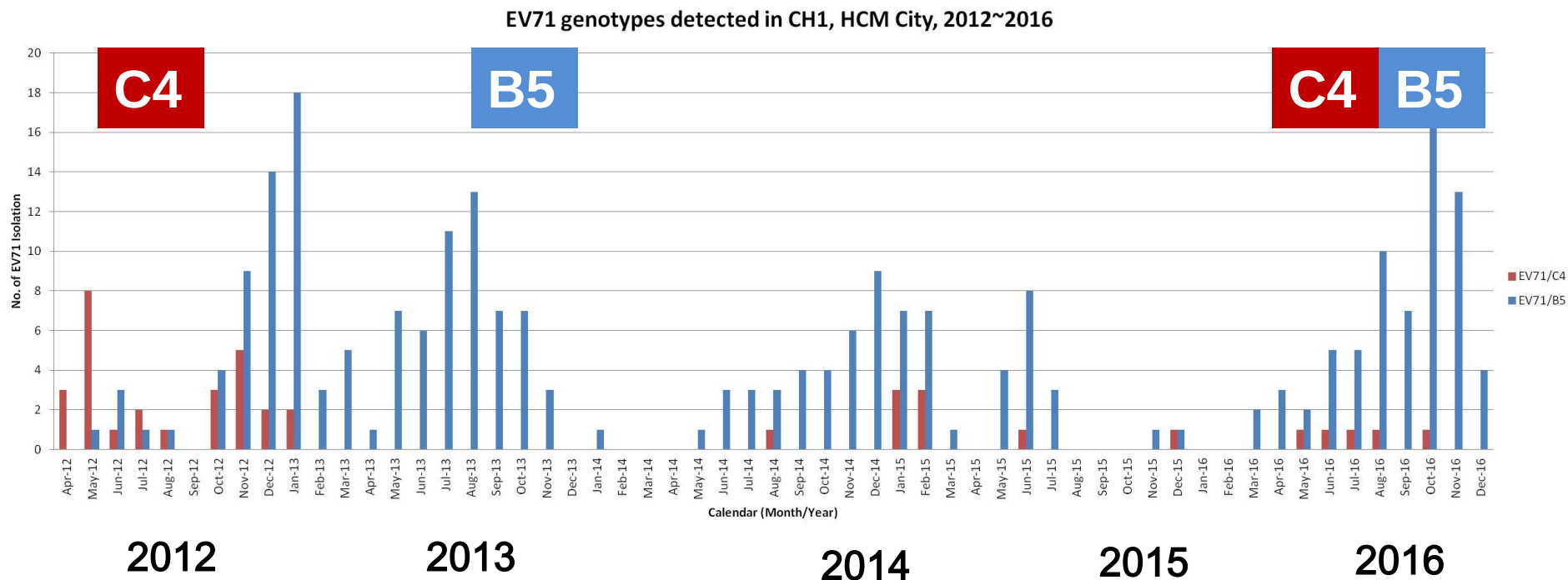
Frozen at -70°C
and shipped to
NHRI, Taiwan

Sera were used to
measure neutralizing
antibody against EV-
A71

Evaluation of two diagnostic methods for enterovirus detection at CH1, HCM City, Vietnam

Year	Virus isolation (CH1, Vietnam)			CODEHOP (NHRI, Taiwan)		
	No. tested	EV Positive (%)	EV-A71 (%)	No. tested	EV Positive (%)	EV-A71 (%)
2011	54	21 (38.9)	16 (29.6)	54	44 (81.5)	29 (53.7)
2012	409	79 (19.3)	28 (6.8)	409	266 (65.0)	60 (14.7)
2013	412	173 (42.0)	66 (16.0)	412	226 (54.9)	83 (20.1)
2014	521	120 (23.0)	14 (2.7)	521	310 (59.5)	35 (6.7)
2015	356	125 (35.1)	34 (9.6)	356	234 (65.7)	39 (11.0)
2016	324	99 (30.5)	17 (5.2)	325	217 (66.8)	74 (22.8)
2017	250	36 (14.4)	4 (1.6)	50	22 (44.0)	2 (4.0)

EV71 genotypes detected at CH1, HCM City, 2012~2016



Distribution of top five enteroviruses detected in pediatric inpatients based on VP1-CODEHOP, CH1, HCMC, Vietnam

Year	No. tested	Top 1 N (%)	Top 2 N (%)	Top 3 N (%)	Top 4 N (%)	Top 5 N (%)
2011	54	EV-A71 29 (55.6)	CV-A6 1 (1.9)			
2012	409	CV-A6 141 (34)	EV-A71 60 (15)	CV-A16 37 (9)	CV-A10 8 (2)	CV-A4 6 (1.5)
2013	412	EV-A71 83 (20)	CV-A10 47 (11.4)	CV-A6 29 (7)	CV-A16 20 (4.9)	CV-A12 9 (2.2)
2014	521	CV-A6 139 (27)	CV-A10 64 (12)	CV-A16 40 (8)	EV-A71 35 (6)	CV-A5 9 (2)
2015	356	CV-A6 82 (23)	CV-A16 46 (13)	EV-A71 39 (11)	CV-A4 20 (5.6)	CV-A2 15 (4)
2016	325	EV-A71 74 (22.8)	CV-A10 67 (20.6)	CV-A16 18 (5.5)	CV-A8 14 (4.3)	CV-A6 10 (3.1)
Vietnam 2012-16	2077	CV-A6	EV-A71	CV-A10	CV-A16	
Taiwan 2008-12	431	CV-A16	CV-A6	EV-A71		

Seroprevalence of EV-A71 Neutralizing Antibody between Vietnam and Taiwan

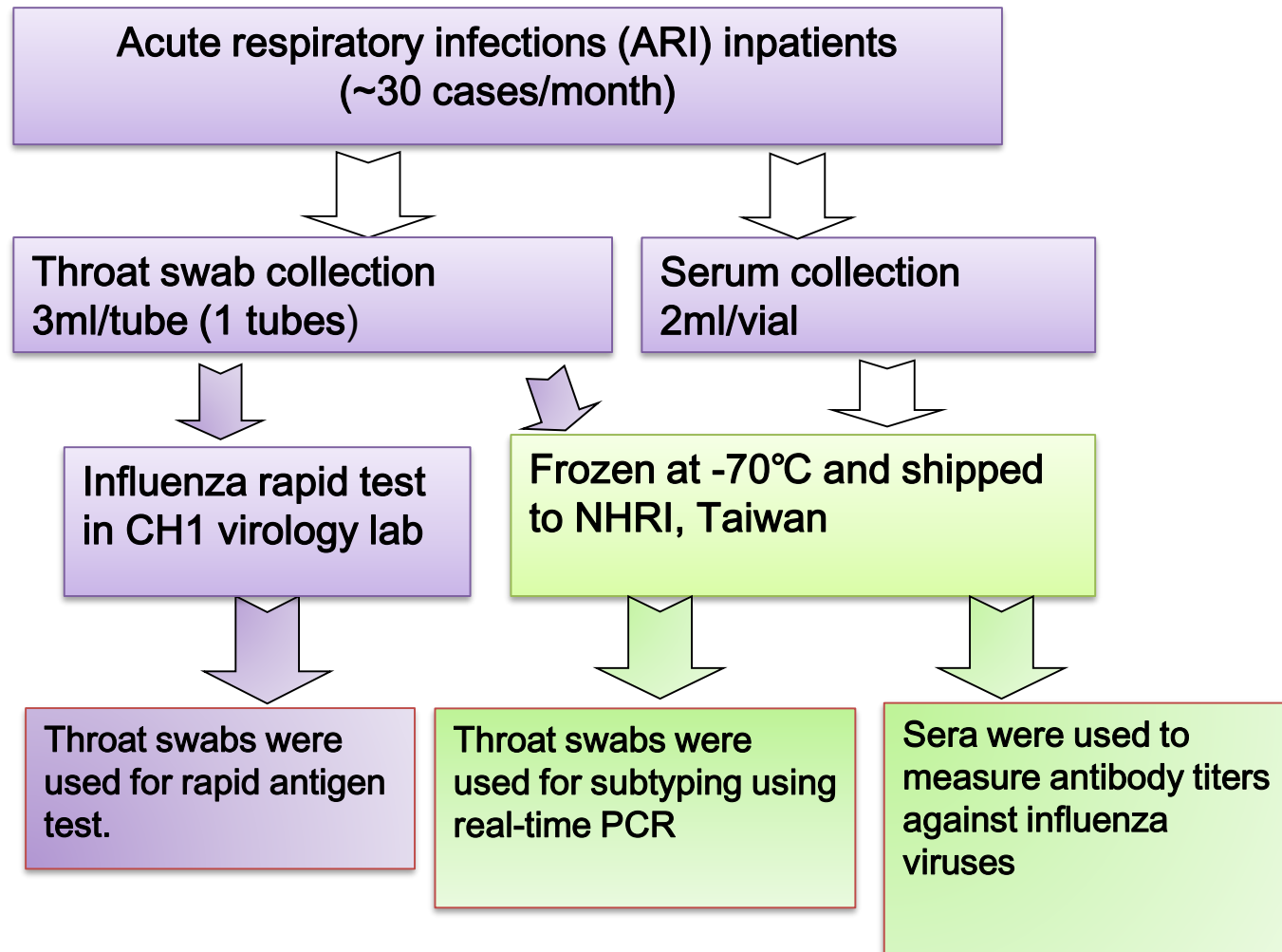
	Vietnam (2012-2013)	Taiwan (1999) <i>(Chiang LY et al., 2007)</i>	Chiayi, Taiwan (2015) <i>(Lee MS et al., unpublished)</i>
Age in Years	Seroprevalence % (n)		
<0.5	15.2 (7/46)	<1%	
0.5–0.9	17.2 (5/29)	10–44	
1–1.9	24.0 (12/50)	0–15	4.0 (2/50)
2–2.9	29.4 (15/51)	11–36	2.0 (1/50)
3–3.9	58.6 (34/58)	15–36	1.8 (1/55)
4–4.9	62.3 (33/53)	34–51	4.3 (2/47))
5–5.9	66.1 (39/59)	56–79	17.9 (5/28)
6–6.9	77.6 (38/49)		25.0 (5/20)
7–7.9	69.2 (36/52)		
8–8.9	85.7 (42/49)		
>9	76.9 (40/52)		



Hospital-based Surveillance of Influenza in HCMC, Vietnam, 2015-2017

Material and Methods

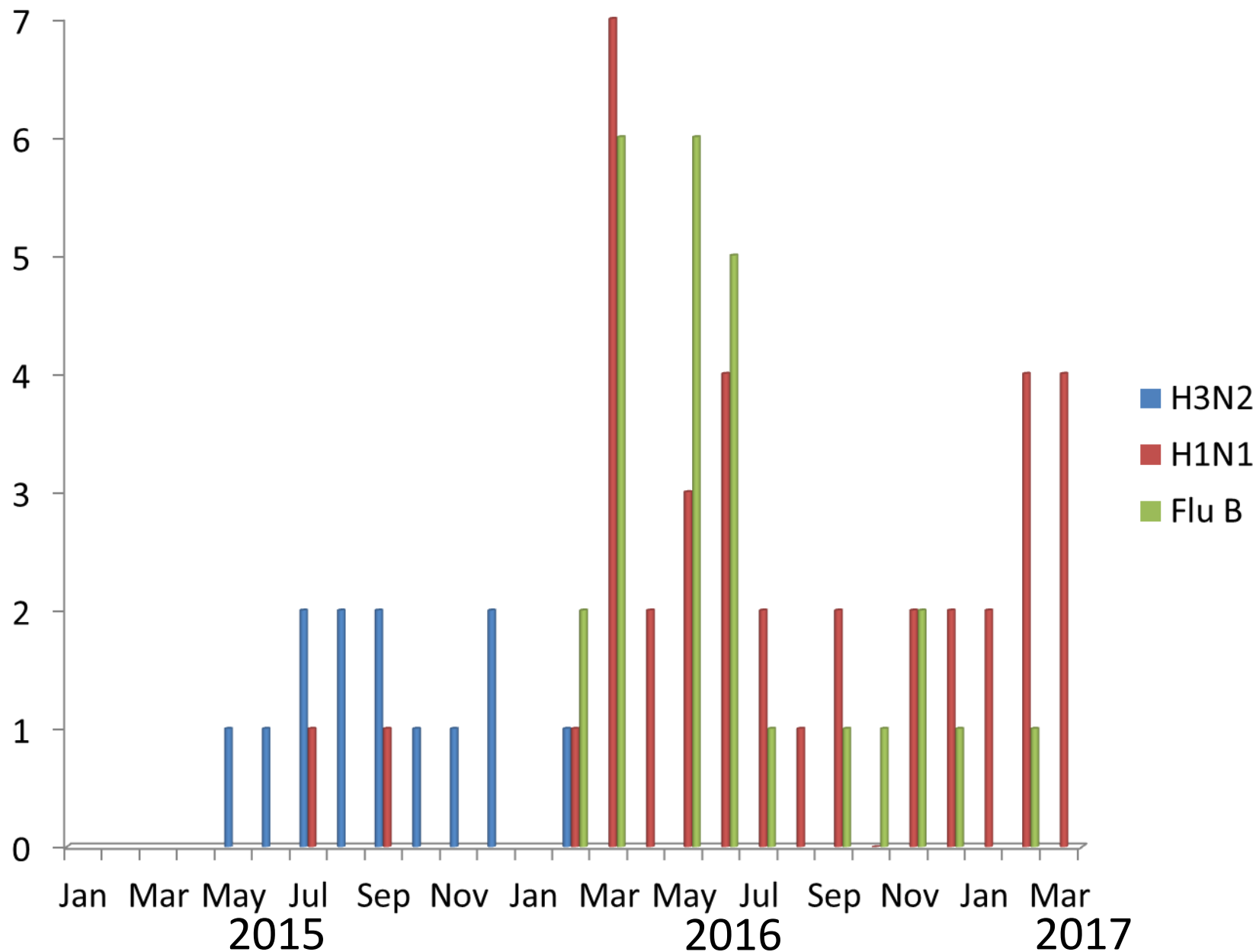
Flowchart of Hospital-Based Influenza Surveillance in Vietnamese Children



Evaluation of two diagnostic methods for influenza detection at CH1, HCM City, Vietnam since 2015

Year	Rapid test (CH1, Vietnam)			Real time PCR (CDC and NHRI, Taiwan)			
	No. tested	Flu A (%)	Flu B (%)	No. tested	H1N1 (%)	H3N2 (%)	Flu B (%)
2015	235	12 (5.1)	0	235	2 (0.9)	11 (4.7)	0
2016	373	24 (6.4)	17 (4.6)	373	26 (6.9)	7 (1.9)	25 (6.7)
2017 Mar	81	10 (12.4)	1 (1.2)	81	10 (12.4)	0	1(1.2)

Influenza viruses detected at CH1, HCMC, 2015-2017



Influenza detection using antigen rapid test at CH1, HCM City, Vietnam, 2017

Month	Negative A/B Test	Positive		Total
		A	B	
01/2017	7	2	0	9
02/2017	49	4	1	53
03/2017	39	4	0	42
04/2017	30	1	0	31
05/2017	32	2	1	35
06/2017	35	2	2	39
07/2017	11	0	1	12
08/2017	37	1	0	38
09/2017	16	1	0	17
10/2017	50	2	1	53
11/2017	11	1	0	12
12/2017	15	0	0	15
01/2018	7	0	0	7
02/2018	7	0	0	7
Total	331	20	6	345

CH1-NHRI Joint Annual Workshop from 2012-2017



2012



2013



2014



2015



2015



2016



2016



2016



2017



2017



2017



2017

Asia-Pacific Network for Enterovirus Surveillance (APNES) Kick-Off, 30 Oct 2017

Founding Members:

1. Cambodia: Pasteur Institute (IPC)
2. Malaysia: University of Malaya
3. Malaysia: University of Malaysia, Sarawak (UNIMAS)
4. Taiwan: National Health Research Institutes (NHRI)
5. Vietnam: Pasteur Institute, HCMC
6. Vietnam: Children Hospital #1, HCMC

Focuses in 2018

1. Sharing information and reagents
2. NGS platform for genome sequencing
3. Developing new intervention tools
4. Identifying funding



Workshop on Regulation of Enterovirus Vaccines in Taiwan and Vietnam, 6-7 March 2018



- Two Taiwanese vaccine companies are planning to conduct multi-center clinical trials of EV71 vaccines in Taiwan and Vietnam

Future Directions

- Facilitate Taiwan vaccine companies to obtain marketing approval of EV71 vaccines in Taiwan and Vietnam
- Collaborating with other organizations for other virus studies and other areas
- Establishing mobile labs (Labs Without Borders)

Labs Without Borders



嘉義縣 3+1 行動醫療服務列車

Medical Screen Trucks in Chiayi, Taiwan



OIE Avian Influenza Sprinter laboratory



Large Truck Labs



BSL-3 laboratory

One container: 29.7 m²

Two containers: 59.4 m²

Three containers: 89.1 m²

Acknowledgement

◆ Funding:

- Ministry of Health and Welfare (MOHW)
- National Flagship Project, Execute Yuan

◆ Partners:

- CH1, HCMC, Vietnam
- Pasteur Institute, HCMC, Vietnam
- Taiwan CDC



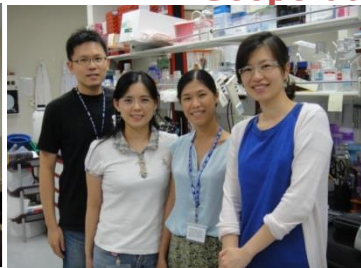
Participating Children and Guardians



Cooperation with CH1 from 2012-2017



2012年至CH1討論EV71計畫



2012年CH1研究助理至國衛院受訓



2012年CH1研究助理至國衛院受訓



2013年討論EV71計畫



2014年與CH1簽署合約



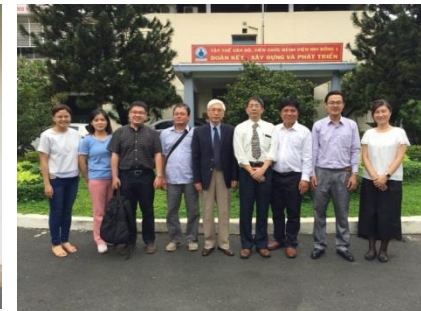
2015年討論EV71實驗問題



2016年討論EV71與流感計畫



2017年討論EV71與蚊媒計畫



2017年討論EV71與流感計畫